Investigation on the Effect of Packing Material, In-package Gas Composition, and Sanitizer on the Safety and Quality of Fresh-cut Produce

By

Natalie Page

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Packaging - Master of Science

2014

ABSTRACT

Investigation on the Effect of Packaging Material, In-package Gas Composition, and Sanitizer on the Safety and Quality of Fresh-cut Produce

By

Natalie Page

Due to safety concerns and increasing demand for high quality fresh-cut produce, there is a communal need to better understand the implications of sanitizing and packaging on fresh-cut produce. The effect of bio-based packaging and non-conventional atmospheres on the quality and safety of chlorine-sanitized celery sticks (CS) inoculated with *Listeria monocytogenes* and stored at 7 ºC for 21 days was investigated. New quantitative descriptive sensory analysis methods were developed to evaluate the color and texture of CS. Additionally, the effect of in-package atmospheres and sanitizers and their interactions on the growth of *Salmonella* Typhimurium, mesophilic bacteria, and yeasts and molds as well as on the physico-chemical and sensorial quality of diced onions (DO) was investigated throughout 14 days of storage at 7 °C. The results show that that the initial gas composition and packaging material both impact the quality and safety of CS. Overall, the combination PLA and 95 kPa O_2 proved most beneficial in maximizing both the safety and quality of CS during one week of storage at 7 ºC. The new texture method involves bending CS until they break and comparing the breakage angle with a reference scale and the new color method involves comparing CS to a Pantone® scale. 2-way interactions between atmosphere and sanitizer that affected *S.* Typhimurium and pH in DO were identified. 3-way interactions (atmosphere, sanitizer, and time) were only observed in the case of headspace CO_2. Elevated CO_2/reduced O_2 and sodium hypochlorite was the best in-package atmosphere and sanitizer combination for enhancing the safety and quality of packaged DO.

I would like to dedicate my thesis to my parents, Larry and Angela Page.

ACKNOWLEDGEMENTS

I would like to express my sincerest gratitude to my advisor, Dr. Eva Almenar for her guidance, patience, unwavering support, and immeasurable time throughout my degree. I would also like to thank my committee members, Dr. Elliot Ryser, Dr. Janice Harte, and Dr. Susan Selke for their valuable insight, advice, and time throughout this process. I am also extremely thankful to Dr. Jaime González-Buesa for teaching me so much and his continuous help throughout my research. I would like to convey gratitude to my fellow graduate and undergraduate students that made this research possible through their knowledge, time, and onion peeling skills. I would like to show my appreciation for the Michigan State University Food Safety Group that awarded me a graduate assistantship, helping to fund my continued education.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. Introduction

Consumer demand for minimally processed fruits and vegetables has increased dramatically over the last ten years due to convenience and consumers increased interest in healthy foods (United Fresh Foundation, 2010). Onions (*Allium cepa* L.) are one of the most widely utilized vegetables in the world, with an annual per capita consumption of nearly 6 kg (The National Onion Association, 2011), and celery (*Apium graveolens* L.) is one of the most desired fresh-cut products due to its taste, texture and low caloric content (United Fresh Foundation, 2010).

As ready-to-use (RTU) produce items, celery and onions have short shelf lives damage and stress during processing can lead to changes in color (Viña and Chaves, 2003; Gómez and Artés, 2005; Viña et al., 2007; Rizzo and Muratore, 2009; Block et al., 1997; Howard et al., 1994; Selman, 1993; Zaulia et al., 2013), pithiness development in celery (Saltveit and Mangrich, 1996; Gómez and Artés, 2005), and loss of turgor (Prakash et al., 2000; Viña and Chaves, 2003; Viña et al., 2007). Moreover, spoilage microorganisms rapidly proliferate due to the easy accessibility to nutrients resulting from processing. In addition to spoilage microorganisms, pathogenic microorganisms are a growing concern of the minimally processed produce industry since they can also be transferred during cutting, washing, or drying stages.

As another analytical tool for assessing fresh produce quality, quantitative descriptive sensory analysis (QDA) allows scientists to identify key sensory attributes of a product (Stone et al., 1974). Evaluations using QDA are based on several types of scales. An anchored 15-point scale method was developed by Poste et al. (1991) for sensory analysis of foods and adapted for celery attributes, which include color, off-aroma, hardness, celery flavor, and off-flavor (Prakash et al., 2000). A nine-point scale technique was developed by Kader et al. (1973) to assess quality attributes of harvested lettuce. This scale has been adapted for analysis of celery sensory

attributes, which include aroma, flavor, texture, color, visual structural integrity and general appearance (Gómez and Artés, 2004; Zhang et al., 2004). A nine-point scale for the assessment of celery pithiness has also been developed (Saltveit and Mangrich, 1996).

Utilizing the Munsell color system is common in QDA (Meilgaard et al., 1999) to identify the color of fresh produce on a given scale. The PANTONE® Company (Carlstadt, New Jersey) has standardized colors for consistent use by applying the Munsell color theory. PANTONE® is internationally recognized as a standard language for communicating colors in a variety of industries (PANTONE, 2013). PANTONE® colors can be used to effectively assess the color changes that occur during the shelf-life of various fresh-cut fruits and vegetables, but have not yet been applied to celery.

Many attributes evaluated by QDA use Spectrum® methodology (Bett, 2002). Some spectrum methods published in Meilgaard et al. (1999) include universal scales that assess texture by crispness, hardness and juiciness. For fruits and vegetables, these attributes are important because they can address the major issues associated with the texture quality. However, these methods require that panelists compress products between their teeth and evaluate it while it is in their mouths. For fresh-cut produce, flexibility is an alternative indicator of freshness and texture changes can be determined by flexing or bending products, however, a standardized scale has not yet been described for training and evaluations for flexibility. Therefore, there is a need to develop a standardized method that allows researchers to assess flexibility by training panelists to bend products.

Various strategies have been developed to better maintain celery freshness. Low temperature storage combined with high CO_2/low O_2 controlled atmospheres can extend the shelf life of celery by several days (Saltveit, 1997; Suslow and Cantwell, 2000; Gómez and Artés,

2004) as can passive modified atmosphere packaging (MAP) (Viña and Chaves, 2003; Gómez and Artés, 2005; Viña and Chaves, 2006; Viña et al., 2007; Rizzo and Muratore, 2009).

However, no information has been published about the benefits from active MAP, in which the package atmosphere is established at the time of sealing, over passive MAP on the shelf life of fresh-cut celery. In addition, the effect of non-conventional active packaging atmospheres, including noble gases, superatmospheric oxygen, and nitrogen, on shelf life extension of fresh-cut produce has been explored for some products (Allende et al., 2004; Rocculi et al., 2005; Escalona et al., 2006; Artés et al., 2009), but not for celery.

Furthermore, MAP with low O_2 levels and/or high CO_2 levels can extend the shelf life of fresh-cut onions by reducing respiration and discoloration, maintaining sucrose content, and preserving aroma (Blanchard et al., 1996; Forney et al., 2012; Liu and Li, 2006; Selman, 1993). The beneficial effect of MAP on shelf life extension of RTU onions has also been proven by sensory panels (Liu and Li, 2006; Blanchard et al., 1996).

Several studies have focused on the microbiological safety of fresh-cut celery (Prakash et al., 2000; Lu et al., 2005; Kwak et al., 2011; Vandamm et al., 2013). However, very little information has been published on *Listeria monocytogenes* in celery, most likely because fresh produce has not been traditionally considered a special risk product (Aguado et al., 2004). Recently, *L. monocytogenes* was identified in ready-to-eat salads containing raw celery (Cordano and Jacquet, 2009) indicating that celery occasionally poses a risk to the consumers. Furthermore, from January to October 2010, ten cases of hospital-acquired listeriosis in Texas (USA), including five deaths, were traced to commercially diced celery that was used as an ingredient in chicken salad (Gaul et al., 2013).

In addition, *Salmonella* spp in onions has been reported as responsible for 8 confirmed outbreaks and 348 illnesses in the past decade (Table 1).

Table 1. Outbreaks and illnesses in the world associated with onions from 2004 to 2012.

Year	Country	Species / Serotype	Total Illnesses	Hospitalizations	Deaths	Food Vehicle	Contaminated ingredient	Source
2004	USA (Multistate)	*Salmonella* Berta	155	16	N/R	Egg, onion, taco beef	beef	1
2004	Australia	*Salmonella* Typhimurium 12a	28	3	0	gourmet rolls/red onion	N/R	2
2005	USA (New York)	*Salmonella* Newport	27	2	0	onion, tomato	root, vine stalk	1
2009	USA (Multistate)	*Salmonella* Javiana	9	N/R	N/R	green onion / scallion	N/R	1
2009	USA (Kansas)	*Salmonella Newport*	33	7	0	sandwich, salad	onion, lettuce	1, 3
2010	USA (Michigan)	*Salmonella* Javiana	41	5	0	potato salad	yellow onion	1
2010	Canada (Ontario)	*Salmonella Oranienberg*	25	N/R	N/R	green onions	N/R	4
2011	Sweden	*Salmonella* Haifa	30	0	0	onion	N/R	5

N/R stands for Not Reported.

[1] CDC's Foodborne Outbreak Online Database (http://wwwn.cdc.gov/foodborneoutbreaks/).

[2] Reported foodborne illness and gastroenteritis in Australia: Annual report of the OzFoodNet network, 2004. The OzFoodNet Working Group (http://www.ncbi.nlm.nih.gov/pubmed/16119765).

[3] Kansas Department of Health and Environment Investigative Reports (http://www.kdheks.gov/epi/outbreaks.htm).

[4] Archived News Release, Ministry of Health and Long-Term Care, Ontario (Canada) (http://news.ontario.ca/mohltc/en/2010/08/salmonella-oranienberg-outbreak-in-ontario.html).

[5] EFSA Panel on Biological Hazards Panel, 2013. Scientific Opinion on the risk posed by pathogens in food of non-animal origin. EFSA Journal 2013; 11(1):3025 (http://www.efsa.europa.eu/en/efsajournal/pub/3025.htm).

Several technologies have been explored as means to reduce spoilage and pathogenic microorganisms in RTU onions including controlled atmosphere (CA) (Blanchard et al., 1996), sanitizing washes (Park et al., 1995), commercial fermentation products (Yang et al., 2011), UV light (Hadjok et al., 2008), modified atmosphere packaging (MAP) (Zaulia et al., 2013), and active packaging (Piercey et al., 2012). Among these technologies, gas compositions (CA and MAP) and sanitizing washes combined, or not, have been most widely investigated. The growth of psychrotrophic microorganisms on chlorine-sanitized diced yellow onions (*Allium cepa* L. cv. Blitz) increased more than 4 log CFU/g in a CA of 2 kPa O_2 + 0 kPa CO_2 but only ~2.5 log CFU/g in a CA of 2 kPa O_2 + 10 kPa CO_2 after 14 days at 4 °C, showing that the enrichment of a low O_2 atmosphere with CO_2 can reduce psychrotrophic proliferation in diced yellow onions (Blanchard et al., 1996).

Fresh-cut produce is commonly marketed in packages made from petroleum-based materials. However, environmental concerns surrounding these packages have created a need for more environmentally-friendly materials (Tharanathan, 2003). Polylactic acid (PLA), a commercially produced bio-based plastic material, is one suitable alternative for packaging of fresh produce (Almenar et al., 2008; Almenar et al., 2010; Joo et al., 2011; Forney et al., 2012). PLA-based packages are clear and highly appealing to consumers (Koutsimanis et al., 2012). Although this material has been previously evaluated for some types of whole and fresh-cut produce, its suitability for fresh-cut celery and onion packaging has not yet been assessed.

The objectives of this study were: (1) to compare the effect of packaging material and initial gas composition on the quality and safety of celery sticks stored at 7 ºC, (2) develop reproducible methods for flexibility and color descriptive sensory assessments for fresh-cut celery, (3) to compare the efficacy of different in-package gas compositions and of several

commercial sanitizing washes on the growth of *S.* Typhimurium, total aerobic bacteria, and yeasts and molds on diced onions, (4) establish interactions between in-package gas compositions and sanitizing washes and their effects on the microbiological safety and quality as well as the physico-chemical quality of diced onions, and (5) to determine the best sanitizer and in-package gas combination for enhancing the microbiological safety and quality of packaged diced onions.

2. Literature Review

Due to changes in consumers' lifestyles over the last decade, the demand for minimally processed produce has notably increased (Fresh Cut, 2008). During this same period of increased processed produce consumption, pathogenic microorganism contamination in RTU produce has also increased. *Salmonella* spp. has been reported as responsible for 3 outbreaks and 184 illnesses (Erickson 2010) in fresh-cut onions. In addition to pathogenic microorganisms, spoilage microorganisms are a growing concern of the minimally processed produce industry due to their increased proliferation caused by the favorable environment resulting from processing. In addition to traditional quality and microbial assays, sensorial analysis is crucial for understanding the human aspect of produce freshness perception and purchasing behavior.

2.1 Means to reduce spoilage and pathogenic microorganisms in processed produce

Technologies such as controlled atmosphere (CA), sanitizing washes, commercial fermentation products, UV light, modified atmosphere packaging (MAP), and active packaging have been explored as means to reduce spoilage and pathogenic microorganisms in processed onions (Blanchard et al., 1996; Hadjok et al., 2008; Yang, Fan, Jiang, Doucette, 2011; Zaulia et al., 2013; Piercey et al., 2012). Additionally, these technologies can affect the sensory quality of processed onions. Among these technologies, CA and MAP with or without a sanitizing wash have most widely been investigated.

Various strategies have also been studied to better maintain celery freshness. Low temperature storage combined with high CO_2/low O_2 controlled atmospheres can extend the shelf life of celery by several days (Saltveit, 1997; Suslow and Cantwell, 2000; Gómez and Artés, 2004) as can passive modified atmosphere packaging (MAP) (Viña and Chaves, 2003; Gómez and Artés, 2005; Viña and Chaves, 2006; Viña et al., 2007; Rizzo and Muratore, 2009).

However, no information has been published about the benefits from active MAP, in which the package atmosphere is established at the time of sealing, over passive MAP on the shelf life of fresh-cut celery. In addition, the effect of non-conventional active packaging atmospheres, including noble gases, superatmospheric oxygen, and nitrogen, on shelf life extension of fresh-cut produce has been explored for some products (Allende et al., 2004; Rocculi et al., 2005; Escalona et al., 2006; Artés et al., 2009), but not for celery.

2.1.1. Effect of gas compositions and sanitizers on spoilage microorganisms

Blanchard et al. (1996) carried out a CA study using diced yellow onions that had been dipped in a 100 ppm chlorine solution for 30 seconds before storage in either: 2% O_2 + 0% CO_2, 2% O_2 + 5% CO_2, 2% O_2 + 10% CO_2, 2% O_2 + 15% CO_2, 20% O_2 + 0% CO_2, or air at 4 °C for 2 weeks. Throughout storage, 2% O_2 + 10% CO_2 had the lowest psychrotrophic growth (~2.5 log CFU/g increase) while onions stored in 2% O_2 + 0% CO_2 or air had the most, with an increase of more than 4 log CFU/g from day 0 to day 14. The storage atmosphere of 2% O_2 + 10% CO_2 also had the best sensory scores, with a score of 8 out of 9 for general appearance, and 7.4 out of 9 for aroma after 14 days. This atmosphere was significantly better than all others in terms of sensory quality, while air was scored the worst (3.1/9 for appearance and 3.3/9 for aroma). This research indicates that a storage atmosphere of 2% O_2 + 10% CO_2 can help to maintain the microbial safety and quality, as well as consumer perceived quality.

Liu and Li (2006) studied the growth of total psychrotrophs and yeasts in sliced onions packaged in LDPE bags containing 40% CO_2 + 1 % O_2 or air during storage at -2, 4, and 10 °C for 2 weeks. The authors found that onions packaged in high CO_2 at 4 °C maintained initial psychrotroph counts (~3 log CFU/g) through day 5, and yeast growth was also maintained, with both high CO_2 and air stored onions at -2 and 4 °C containing ~4 log CFU/g of growth at day 7.

Thus calling attention to the fact that storage temperature is a more influential parameter than in-package atmosphere for preventing yeast growth in sliced onions.

In diced red onions sanitized with peracetic acid and packaged in sealed polyethylene (PE) bags, sealed polylactic acid (PLA) containers, or non-sealed PLA containers for 3 weeks at 4.5 °C, aerobic plate counts increased from 3.87±0.12 log CFU/g to 7.74, 6.82, and 8.21 log CFU/g after 12 days in the sealed PE bags, sealed PLA containers, and non-sealed PLA containers, respectively. The in-package CO_2 accumulation, 15, >25, and 4% for the sealed PE bags, sealed PLA containers, and non-sealed PLA containers, respectively, directly correlated with the reduction of aerobic microorganisms, indicating that high in-package CO_2 concentrations can help to improve microbial shelf life of red diced onions at 4.5 °C (Forney et al., 2012).

Selmen (1993) reported that diced onions packaged in OPE/PE bags that were either perforated or contained modified atmosphere showed varying shelf lives depending on atmosphere and storage temperature. Diced onions experienced no discoloration when packaged in air for 14 days at 5 °C and 11 days at 10 °C. Packages containing 10% O_2 + 5% CO_2 helped to maintain onion quality for 1 week at 5 °C and 5 days at 10 °C, while perforated (control) bags only allowed for 5 days at 5 °C and 2 days at 10 °C. In polyvinylchloride bags sealed with air, diced onions experienced no discoloration for 7 days at 5 °C and 3 days at 10 °C indicating that not only is temperature and in-package atmosphere important, but also packaging material for maintaining typical onion color during storage.

A similar passive atmosphere packaging and chlorine sanitizing technique was studied by Park and Lee (1995) with hand-diced onions in polystyrene trays wrapped with linear low density polyethylene film for storage at 5 °C for 3 weeks. Diced onions were dipped into a 25 °C

sanitizing solution containing either 0 (control), 30, 50, or 100 ppm chlorine for 1 minute. Mesophilic populations were reduced to approximately 0.5 log CFU/g at the initial sampling for all samples treated with chlorine, while the control contained populations of nearly 2 log CFU/g. There were no differences between sanitizer concentration and all treatments exceeded 8 log CFU/g at the end of storage. The authors also observed that the surface color of the diced onions did not vary between treatments throughout storage. Because there were no observed differences between microbial populations or onion color over time, the minimum 10 ppm chlorine sanitizer was determined to be sufficient for reducing initial microflora. This study helps to point out the need for more innovative methods to reduce microbial populations in diced onions throughout their storage to improve overall marketability.

Park and Lee also (1995) researched the effect of varying concentrations of chlorine sanitizing wash on the initial total microbial populations, ascorbic acid content, and color of diced onions. Similar to the previously mentioned packaging study, onions washed with 10, 30, 50, and 100 ppm chlorine at 25 °C showed little to no differences in the measured parameters. The naturally occurring microbial populations were reduced from 1.7 log CFU/g to < 1 log CFU/g for all concentrations, with only a slight destruction (0.3 mg/100g) of ascorbic acid in onions washed in 10 ppm chlorine, again indicating that it is an adequate choice for an initial reduction of microorganisms while maintaining quality.

The quality and bacterial populations of diced yellow onions with passive MAP and an ethylene absorber were studied by Howard et al. (1994). Onions were first diced by hand with a knife and then submerged in a 100 ppm chlorine solution before being packaged in PE bags and stored at 2 °C for 10 days. Packages with and without an ethylene absorber reached a CO_2 concentration of 7% after 10 days. Differences were observed, however, for mesophilic bacterial

populations, starting on day 3 of storage. Onions packaged with an ethylene absorber reached a growth level of 7 log CFU/g, while those without the absorber were less than 6 log CFU/g on day 10. These results indicate that the naturally accumulating ethylene inside the diced onion package is beneficial to reduce the growth of bacterial microorganisms during storage, even at low temperatures such as 2 °C.

Beerli et al. (2004) researched the effect of hydrogen peroxide (H_2O_2) and sodium dichloroisocianurate (NaDCC) sanitizers on the microbial and physico-chemical quality of sliced onions for 1 week at 4 °C. Onion slices treated with H_2O_2 (4 and 6%) contained lower aerobic mesophiles and yeast and mold populations than NaDCC (50 and 100 ppm), however there were not differences between the two sanitizers for aerobic psychrotrophs, total coliforms at 35 °C, total soluble solids, and weight loss. While the NaDCC treated onions experienced an increase in pH, H_2O_2 treated onions increased in firmness during storage, indicating that while some sanitizers can help to reduce microbial populations, they can also damage sliced onions, producing undesirable affects.

2.1.2. Effect of gas compositions and sanitizers on pathogenic microorganisms

Several studies have focused on the microbiological safety of fresh-cut celery (Prakash et al., 2000; Lu et al., 2005; Kwak et al., 2011; Vandamm et al., 2013). However, very little information has been published on *Listeria monocytogenes* in celery, most likely because fresh produce has not been traditionally considered a special risk product (Aguado et al., 2004). Recently, *L. monocytogenes* was identified in ready-to-eat salads containing raw celery (Cordano and Jacquet, 2009) indicating that celery occasionally poses a risk to the consumers. Furthermore, from January to October 2010, ten cases of hospital-acquired listeriosis in Texas

(USA), including five deaths, were traced to commercially diced celery that was used as an ingredient in chicken salad (Gaul et al., 2013).

Farber et al. (1997) investigated the effect of passive MAP in combination with chlorine sanitizer on the growth of *Listeria monocytogenes* on inoculated sliced onions. Whole peeled onions were dipped in a 200 ppm sodium hypochlorite solution for 10 seconds before being sliced. Onion slices were then inoculated with *L. monocytogenes* and stored in sealed OPE bags at 4 and 10 °C for 9 days. In-package CO_2 accumulation was 23% in bags stored at 4 °C and 52% in bags at 10 °C on day 9. There was no increase in *L. monocytogenes* growth in onion slices stored at 4 °C, likely because *Listeria* does not grow well at low temperatures. There was a slight (~1 log CFU/g) increase in *L. monocytogenes* populations from day 0 to day 9 in onions at 10 °C. The unexpectedly low increase of *L. monocytogenes* during the 9 day storage period can be attributed to the large buildup of CO_2 inside the bags, preventing *Listeria* proliferation.

Sy et al. (2005) applied gaseous chlorine dioxide (ClO_2) to whole Vidalia onions to determine the possible reductions in *Salmonella* and yeast/mold growth for the produce. *Salmonella enterica* surface inoculated onions were treated with ClO_2 in concentrations of 0 (control), 1.4, 2.7, and 4.1 mg/L, resulting in reductions of 0 (control), 0.83, 1.89, and 1.94 log CFU/piece, respectively. Yeast and mold growth was also reduced on the onion surface after ClO_2 treatment, with reductions of 0.36 and 0.22 log CFU/piece for 1.4 and 2.7 mg/L treatments, and an increase of 0.22 log CFU/piece for those treated with 4.1 mg/L ClO_2. This study illustrates that chlorine dioxide is a viable alternative sanitizing option for onions, but that the proper concentration must be utilized to ensure that the onions are not damaged, allowing for increased yeast and mold growth.

2.1.3. Non-traditional means to reduce microbial populations on minimally processed produce

As an alternative to immersion sanitizing techniques, Hadjok et al. (2008) applied ultraviolet (UV) light combined with hydrogen peroxide (H_2O_2) to sliced Spanish onions to reduce both surface and internal pathogenic microorganisms. The onion slices were submerged in 500 mL of inoculum containing 7 log CFU/g of *Salmonella* Montevideo for 20 minutes before being vacuumed cyclically to induce internalization of the bacteria. Each side of the onion slice was treated with 50 °C 1.5% v/v H_2O_2 mist while exposed to a UV lamp (254 nm) for 1 minute while control samples were treated with a 200 ppm calcium hypochlorite solution for 3 minutes. *Salmonella* populations present on the surface of the slices were reduced 3.66 log CFU/g while the calcium hypochlorite control was only reduced 0.34 log CFU/g. Internalized *Salmonella* were reduced 0.97 log CFU/g when treated with UV/H_2O_2, and 0.05 log CFU/g for the control, indicating that this experimental sanitizing technique is significantly more effective at reducing surface populations of *Salmonella* Montevideo than calcium hypochlorite on sliced onions. While the observed surface *Salmonella* reduction is notable, studying the additional effect of storage time on microbial populations is necessary to deem UV/H_2O_2 as a viable alternative sanitizing technique for sliced onions.

Purple Brazilian onions were sliced and diced in a study by Berno et al. (2014) to determine the effect of storage temperature on the quality of fresh-cut onions. Hand-sliced or diced onions were kept in polypropylene snap fit containers at 0, 5, 10, or 15 °C for up to 15 days. Sliced onions were darker (higher luminosity) than diced onions from day 0, and better maintained their luminosity, Chroma, and Hue values throughout the storage study compared to diced onions. Diced onions experienced greater surface color changes than sliced onions at all temperatures, likely due to the increased surface area to volume ratio produced by the dicing

action. However, for all color attributes reported, onions (both sliced and diced) kept at 5 °C best maintained their initial color compared to the other storage temperatures studied. This study helps to stress the importance of finding better storage conditions that can help to mitigate the loss of initial quality due to the processing conditions necessary for fresh-cut onions.

Although the effect of modified atmosphere packaging and assorted sanitizers on spoilage and pathogenic microorganisms in minimally processed produce has been studied, there is a lack of information on sanitizer and packaging material on pathogenic microorganism growth in fresh-cut celery, lacking available methods for flexibility and color descriptive sensory assessments for fresh-cut celery, and no information on the effect of interactions between in-package gas compositions and sanitizers in regards to the microbiological safety and quality. In addition, there is no information on the effect of either in-package gas compositions or sanitizing washes on *Salmonella* Typhimurium growth on minimally processed onions.

3. Effect of non-conventional atmospheres and bio-based packaging on the quality and safety of *Listeria monocytogenes*-inoculated fresh-cut celery (Apium graveolens L.) during storage

3.1. Materials and methods

3.1.1. Celery

Celery (*Apium graveolens* L.) was purchased from a local distributor (Stan Setas Produce, Lansing, MI, USA), transported under refrigeration to the MSU School of Packaging, maintained at 7 ºC and used within 2 d of delivery. Celery was visually inspected, selected, and minimally processed with a stainless steel knife (Granton, Sheffield, UK) into 10 cm long sticks (~ 20 g each). After processing, the celery sticks were rinsed with cold tap water and stored inside a temperature controlled chamber at 7 ºC overnight. The following day, the celery sticks were immersed in a commercial sanitizer containing 50 mg L^{-1} available chlorine at pH 7.5 (XY-12, Ecolab, St. Paul, MN, USA) for 1 min at 7 ºC, centrifuged for 1 min using a batch spin dryer (SD50 LT, Heinzen Manufacturing Intl., CA, USA) and packaged. The celery sticks were not rinsed after sanitizing, following the current processing conditions of celery processors in the United States, meaning some residual chlorine could be present after processing. The sticks intended for *L. monocytogenes* inoculation were treated as described in Section 8.2.7.

3.1.2. Packaging

Two packaging films were used: A 51 µm-thick film composed of polypropylene (PP) and low density polyethylene (LDPE) (commonly used in fresh-cut produce packaging in the U.S.) and a 44-µm thick film made of polylactic acid (PLA) coated with high molecular weight PLA (EVLON EV-HS1, BI-AX International Inc., Wingham, ON, Canada). Codes used for the petroleum-based polyolefin and for the bio-based polyester from now on are PP/PE and PLA, respectively.

3.1.2.1. Packaging and storage

Both packaging films were formed into 20 cm length × 15 cm width bags using an impulse sealer (Ceratek, Sencorp Systems Inc., Hyannis, MA, USA). An amount of 10-12 celery sticks were placed in each bag (≈190 grams per bag). Half of the PLA and PP/PE bags were sealed without atmosphere replacement for passive modified atmosphere packaging (MAP) (A-PLA and A-PP/PE). The air in the remaining PLA bags was replaced with 95 kPa O_2 + 5 kPa N_2 (O_2-PLA), 99 kPa N_2 + 1 kPa O_2 (N_2-PLA), or 6 kPa O_2 + 12 kPa CO_2 + 82 kPa N_2 (CO_2-PLA) using a Multivac flusher (Model A300/16, Sepp Haggenmüller KG, Wolfertschwenden, Germany) for the active MAP treatments. As a control, celery was packaged under the same conditions as in the passive MAP treatment, but the bottom of the bags was partially cut off, creating a non-modified atmosphere package (coded as open bags). All packages were stored at 7 ºC until the day of testing.

3.1.2.2. Packaging material characterization

Oxygen, carbon dioxide, water vapor and ethanol transmission rates were assessed for PLA and PP/PE. Oxygen transmission rate (OTR) was determined at 23 °C and 0% RH according to the American Society for Testing Materials (ASTM) method D3985 (ASTM, 2005) using an OX-TRAN® Model 2/21 (MOCON, Minneapolis, MN, USA). Carbon dioxide transmission rate (CO_2TR) was measured using a PERMATRAN-C™ Model 4/41 (MOCON) under the same conditions. Water vapor transmission rate (WVTR) was determined at 23 °C and 100% RH according to ASTM method F1249 (ASTM, 2006) using a PERMATRAN-W™ Model 3/33 (MOCON). Permeation cells made from stainless steel were used to gravimetrically determine the ethanol transmission rate for PP/PE and PLA using a slightly modified ASTM method E96-80 (ASTM, 1980). All testing was conducted in triplicate.

In all cases, the permeability coefficients (kg m m^{-2} s^{-1} Pa^{-1}) were obtained as follows:

$P = (TR \times l)/\Delta p$

where *TR* is the transmission rate value (kg m^{-2} s^{-1}), *l* (m) is the film thickness, and Δp is the partial pressure differential across the film (Pa). The permselectivity coefficient (β; ratio of CO_2 to O_2 permeation) was also calculated.

3.1.3. Atmosphere composition

Progressive changes in CO_2, O_2, and N_2 were monitored in all packages using a gas chromatograph (Thermo Scientific Trace GC Ultra, Thermo Electron S.p.A., Rodano, Italy) equipped with a thermal conductivity detector and a Carboxen 1010 Plot capillary column, 30 m in length with a film thickness of 30 µm, and an internal diameter of 0.53 mm (Supelco, Bellefonte, PA, USA). Helium was used as a carrier gas (3 mL min^{-1}). Using a syringe (SGE, Austin, TX, USA), a 100-µL headspace sample was collected through a silicone septum attached to each package. Initial gas compositions were determined to verify correct flushing by the Multivac machine. Subsequently, CO_2, O_2, and N_2 levels were monitored after 1, 3, 5, 7, 10, 14, and 21 d. New bags were used for each analysis day.

Ethanol concentration was determined after 3, 7, 14, and 21 d by injecting 1 mL headspace samples into a gas chromatograph equipped with a flame ionization detector (Hach Carle Series 100 AGC, Loveland, CO, USA) fitted with a 2 m long, 2 mm internal diameter stainless steel column packed with Chromosorb OV-103, 60/80 mesh (Altech Associates Inc., Deerfield, IL, USA). Ethanol content was expressed as microliters of ethanol per liter (µL L^{-1}).

3.1.4. Texture

A texture analyzer (TA-XT2i, Stable MicroSystem, Godalming, UK) equipped with a 50 kg-load cell was used to determine celery stick firmness and toughness. The assays were

performed using a 3-point bending probe (constant span length of 4 cm acting perpendicularly to the celery), which was positioned horizontally with the outer convex surface upwards, centered on the supports, and the pressing force was applied vertically to the middle of the celery. Each stick was flexed at a constant speed of 2 mm s^{-1} until failure. Firmness (N) was calculated as the measured force at 5 mm of the load-displacement curve. Toughness (J m^{-3}) was defined as the area below the stress-strain curve. For texture measurements, all sticks (≈10 per bag, ≈ 60 sticks per treatment) were analyzed.

3.1.5. Color

Celery color was determined after 0, 3, 7, 14, and 21 d of storage using a Minolta CR300 colorimeter (Osaka, Japan) calibrated with a standard white plate (Y=93.84; x=0.3132; y=0.3191) and set with a C illuminant, 2º observer. Readings taken from both the outer convex surface and cut ends were expressed as L*, a* and b* parameters. Hue angle (hº) was calculated using the a* and b* parameters ($h^o=\tan^{-1}(b^*/a^*)$). For color measurements, all sticks (≈10 per bag, ≈ 60 sticks per treatment) were measured at two points on the surface, and at the two cut ends.

3.1.6. Weight Loss

After 21 d of storage, weight loss was determined for all celery stick treatments (6 bags/treatment) by subtracting the final weight from the initial weight with the results expressed as % weight loss.

3.1.7. Preparation of inoculum, inoculation of samples, and microbiological analysis

Stock cultures of three avirulent strains of *Listeria monocytogenes* (J22F, M3, J29H) were maintained at -80 °C and subcultured twice (24 h/37 °C) in 200 mL of trypticase soy broth (Difco, Becton Dickinson, Sparks, MD, USA) containing 0.6% (w/v) yeast extract (Difco,

Becton Dickinson) (TSB-YE). Cultures were combined in equal volumes and diluted 1:100 in tap water (~15 °C) to contain 7.19±0.13 log CFU/mL. The bacterial population in the final cocktail culture was confirmed by appropriately diluting in sterile 0.1% phosphate buffer solution and plating on Modified Oxford Agar (Neogen Corporation, Lansing, MI, USA) (MOX) plates. These plates were then incubated at 37 ºC for 48 h prior to enumeration. The celery sticks were then inoculated by immersion for 30 min to obtain 4.35±0.33 log CFU/g. After centrifugal drying and 18–22 h of storage at 4 ºC, the celery sticks were immersed in 60 L of tap water containing 50 mg L^{-1} available chlorine (XY-12, Ecolab, USA) for 1 min, centrifugally dried and then packaged in both PLA and PP/PE bags in a glove chamber (Labconco 50004 Fiberglass Glove Box, Kansas City, MO, USA) using the gas compositions described in section 2.2. The chamber was used instead of the Multivac machine due to the special safety precautions required for handling the *L. monocytogenes*. Additional PLA-packaged, passive modified atmosphere samples were prepared using unsanitized inoculated fresh-cut celery (immersed in 60 L of tap water).

Following 0, 1, 3, 5, 7, 10, 14, and 21 d of storage at 7 °C, two celery stick samples (~50 g) from each package were diluted 1:4 in Difco Neutralizing Buffer (Becton Dickinson, Sparks, MD, USA) and homogenized in a stomacher (Stomacher 400 Circulator, Seward, Worthington, UK) at 260 rpm for 1 min. Samples were either appropriately diluted in sterile 0.1% phosphate buffer solution and plated on MOX, or filtered using 0.45 μm membrane filters (Millipore, Millipore Corporation, Billerica, MA) and placed on 60 mm diameter petri plates containing MOX for quantification of *L. monocytogenes* after 48 h of incubation at 37°C.

3.1.8. Statistical analysis

All of the experiments were conducted in triplicate and data was expressed as the means ± standard errors. Three replications consisting of two bags each (a total of 6 bags) were analyzed per treatment on each day of testing for quality analysis. For *Listeria* analysis, one bag for each of the three replicates was used (a total of 3 bags). Data were subjected to statistical analysis using a statistical software package IBM SPSS version 19 (IBM Corporation Software Group, Somers, NY, USA). One-way analysis of variance (ANOVA) and Duncan multiple range tests at a significance level $P < 0.05$ were used, except for the microbiological and texture analyses, where comparison of means was conducted with the least significant difference (LSD) test ($P < 0.05$).

3.2. Results and discussion

3.2.1. Packaging material characterization

O_2, CO_2, water vapor and ethanol permeability coefficients of PLA and PP/PE are shown in Table 2. The materials had different permeabilities to the gases and vapors tested. The O_2 and CO_2 permeabilities of PLA were 4 and 5 times lower than those of the PP/PE, respectively. Given that a low permeability is necessary to reduce gas exchange and maintain the flushed gas inside package, PLA could be a viable bio-based material to maintain desired gas compositions in active MAP. The CO_2 and O_2 permeability coefficients of PLA were very similar to those reported for oriented PLA sheets ($33\pm8 \times 10^{-18}$ kg m m^{-2} s^{-1} Pa^{-1} and $4\pm0 \times 10^{-18}$ kg m m^{-2} s^{-1} Pa^{-1}, respectively) by Joo et al. (2011). The ethanol permeability of PLA was lower than that of PP/PE. Information on the permeability of plastics to ethanol is very limited (Robertson, 2013), and no data on the permeability of PLA to ethanol has been found in the literature. The ethanol permeability coefficient of LDPE has been reported as 3.24×10^{-17} kg m m^{-2} s^{-1} Pa^{-1} (Robertson,

2013). This value is higher than our value for PP/PE, which indicates that PP is a better barrier to ethanol than LDPE. In agreement, Scully (2009) reported that LDPE is not as good a barrier to ethanol as PP. The water vapor permeability coefficient of PP/PE was 20 times lower than that of PLA. Our PLA results are in agreement with those reported by Almenar and Auras (2010) under the same analysis conditions (11.98×10^{-15} kg m m^{-2} s^{-1} Pa^{-1}). The O_2, CO_2, and water permeability values of PP/PE were between those reported for LDPE and PP (Almenar and Auras, 2010).

Table 2. Carbon dioxide, oxygen, water and ethanol permeability coefficients for PLA and PP/PE films.

Material	**Permeability coefficient**				**β**
	CO_2 [a]	**O_2 [a]**	**H_2O [b]**	**Ethanol [a]**	**(P_{CO2}/P_{O2})**
PLA	30.34±9.07	5.67±1.17	21.86±3.22	0.10±0.03	5.35
PP/PE	113.4±13.8	28.35±4.61	1.08±0.24	0.51±0.01	4.00

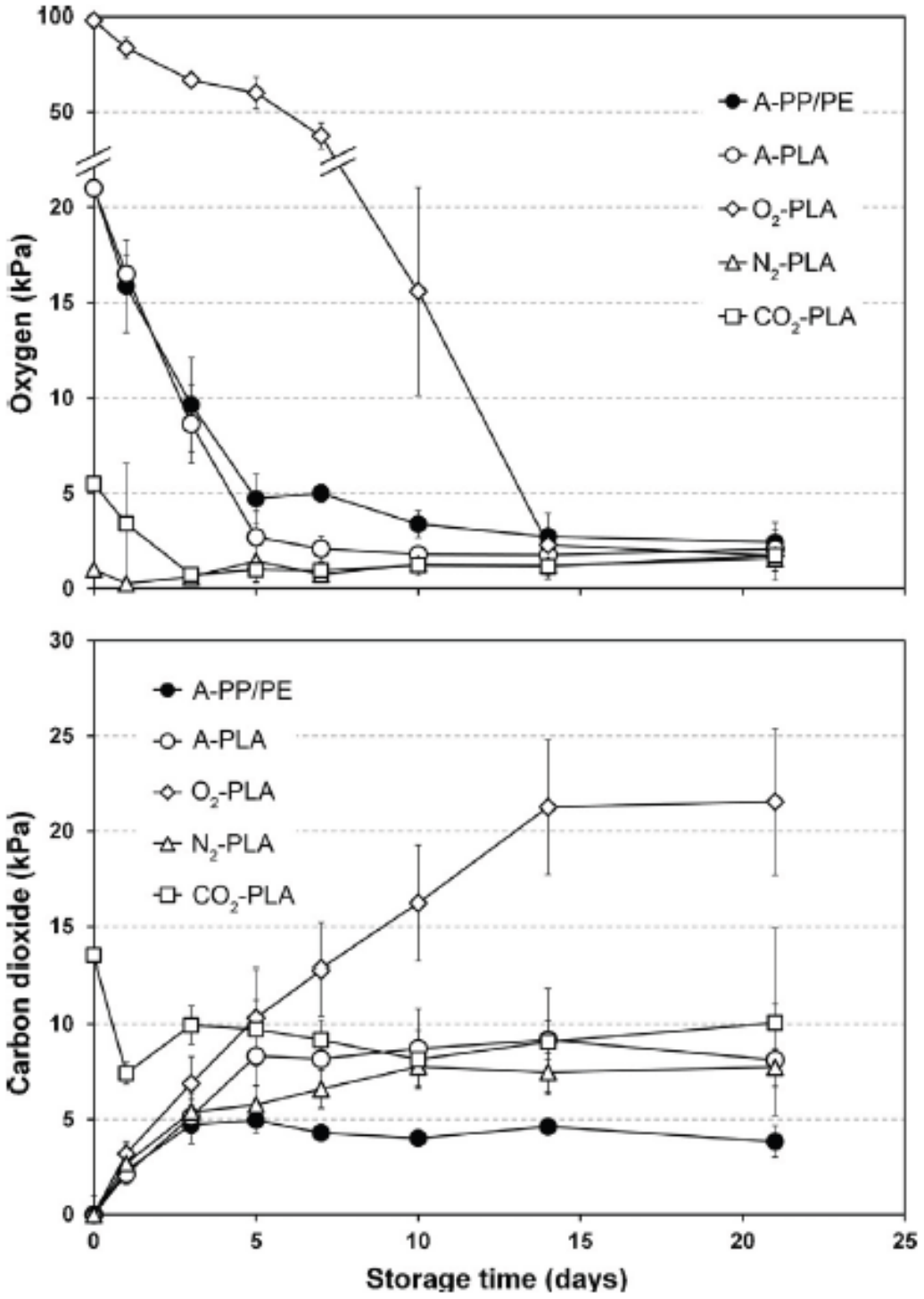
[a] $\times 10^{-18}$ kg m m^{-2} s^{-1} Pa^{-1}
[b] $\times 10^{-15}$ kg m m^{-2} s^{-1} Pa^{-1}

3.2.2. Atmosphere composition

The steady state atmospheres achieved in the A-PLA, N_2-PLA, and CO_2-PLA bags ranged between 1-2 kPa O_2 and 8-10 kPa CO_2 (Figure 1) with these values close to the atmospheres recommended by Gómez and Artés (2004) for shelf life extension of celery during refrigerated storage. The steady state atmospheres reached in the O_2-PLA bags was 2 kPa O_2 and 22 kPa CO_2 (Figure 1). The steady state atmosphere was achieved faster in CO_2-PLA (3 d) than A-PLA and N_2-PLA bags (7 d). Levels of CO_2 and O_2 in the O_2-PLA bags stabilized after 14 d with the A-PP/PE bags reaching steady state after 10 d of storage (2.5 kPa O_2 and 4 kPa CO_2). Bags made from PLA equilibrated faster than those made from PP/PE due to the greater

permselectivity of the PLA (5.35 and 4.00 for PLA and PP/PE, respectively) resulting from their different permeability to O_2 and CO_2 (Table 2). Therefore, PLA would be preferred over PP/PE for fresh-cut celery. Gas evolution in the A-PP/PE bags was very close to that obtained by Gómez and Artés (2005) but non-optimal when celery sticks were packaged in 25 µm LDPE bags and stored at 4 ºC. These same authors found that the higher CO_2 (7 kPa) and lower O_2 (5 kPa) levels achieved in 35 µm OPP bags worked better to maintain fresh-cut celery quality. The relatively higher CO_2 concentration reached in the bags with high O_2 can be attributed to a more stable respiration rate of the celery since O_2 was not a limiting factor during the first 14 d of storage. However, an increased respiration rate due to tissue damage (Cantwell and Suslow, 2002; Martínez et al., 2005; Iqbal et al., 2008), microbial growth (Jacxsens et al., 2003; Artés-Hernandez et al., 2007; Silveira et al., 2008), or headspace volume reduction could be contributing to CO_2 accumulation for this treatment.

Figure 1. Oxygen and carbon dioxide contents of celery stick packages for different materials and initial gas composition s stored at 7 ^{o}C.



Throughout storage, the headspace for PP/PE bags yielded far lower ethanol levels compared to PLA bags age which is most likely due to the 5-fold greater ethanol permeability of

the PP/PE film which favored the escape of ethanol from the package headspace (Table 3). Comparing the different in-package gas compositions, faster ethanol accumulation was observed in the CO_2-PLA and N_2-PLA bags due to their low level of O_2 (~ 1 kPa) (Table 3) which led to earlier hypoxia. Many vegetables generate ethanol through fermentative metabolism at O_2 levels < 2 kPa. Since ethanol was not detected in the A-PLA and O_2-PLA bags until day 14, the packages remained sufficiently aerobic to minimize fermentation for at least a week ($P < 0.05$). The ethanol content in all PLA bags increased from week 2 to 3 with the greatest increase seen in O_2-PLA bags, likely due to the higher CO_2 content. High levels of CO_2 alone (> 20 kPa CO_2) or in combination with low levels of O_2 (> 20 kPa CO_2 and < 2 kPa O_2) have been related to the accumulation of ethanol due to fermentation (Jacxsens et al., 2001). A sensory evaluation is needed to determine the impact of ethanol concentration on consumer acceptance of packaged celery sticks.

3.2.3. Weight loss

The weight of celery sticks in open bags decreased more than 30% after 21 d of storage at 7 ºC (Table 3), showing the need for hermetically sealed containers during the postharvest period. Significantly greater ($P < 0.05$) weight loss was seen after 21 d of storage using PLA (~ 4%) compared to PP/PE bags (< 1%) (Table 3). The higher weight loss of the celery sticks in the PLA bags was due to the higher water vapor permeability coefficient of PLA ($21.86 \pm 3.22 \times 10^{-15}$ kg m m^{-2} s^{-1} Pa^{-1}) compared to PP/PE ($1.08 \pm 0.24 \times 10^{-15}$ kg m m^{-2} s^{-1} Pa^{-1}). The initial atmosphere did not affect celery weight loss since similar weights were obtained for all PLA-packaged celery at the end of the storage.

Table 3. Weight loss (%) and ethanol content (μL L^{-1}) for fresh-cut celery in packages differing in initial gas composition and packaging material during storage at 7 ºC.

Packaging code	Storage time (days) 3	7	14	21	
	Ethanol	**Ethanol**	**Ethanol**	**Ethanol**	**Weight loss**
Open Bags	-	-	-	-	30.99±12.42
A-PP/PE	0.00±0.00 [a*]	0.06±0.11 [a]	0.32±0.25 [a]	0.39±0.28 [a]	0.73±0.77
A-PLA	0.00±0.00 [a]	0.73±0.98 [ab]	12.92±8.94 [ab]	16.34±17.41 [ab]	4.50±0.14
O_2-PLA	0.00±0.00 [a]	0.13±0.16 [a]	12.99±6.80 [ab]	47.88±22.89 [c]	4.67±0.57
CO_2-PLA	8.15±9.98 [b]	6.32±5.42 [ab]	16.31±11.81 [b]	31.97±29.17 [bc]	4.53±0.33
N_2-PLA	10.72±6.25 [b]	7.66±4.93 [b]	17.59±13.55 [b]	19.99±22.36 [abc]	4.87±0.40

[*]Each value represents the mean of three replicates ± standard deviation. Different letters indicate significant differences ($P < 0.05$) between treatments for each time point.

Celery packaged in PLA bags exhibited a > 4% weight loss after 21 d, however, this was less than the maximum 10% water loss considered as a limit of acceptance for celery (Robinson et al., 1975). Therefore, celery sticks in PLA bags would likely remain acceptable for at least 21 d with PLA performing similarly to other plastics. Viña and Chaves (2003) reported a weight loss of 6.2% for minimally processed celery packaged in polyvinylchloride film-covered polystyrene trays after 28 d of storage at 10 ºC. Assuming a linear relationship between weight loss and time, our results for celery sticks stored in PLA at 7 ºC are very similar to those of Viña and Chaves (2003) after 21 d, although their water vapor transmission rate for polyvinyl chloride (4.63×10^{-7} kg m^{-2} s^{-1}) was 2.8 times lower than that of our PLA ($13.08 \pm 1.92 \times 10^{-7}$ kg m^{-2} s^{-1}).

3.2.4. Color

The surface color (hue angle) of celery sticks was affected by both the packaging material and gas composition (Table 4). At the end of storage, celery packaged in A-PLA was greener (higher hº) than that packaged in A-PP/PE ($P < 0.05$) with the former maintaining the green color of celery throughout storage. The difference was likely due to the higher CO_2 level in A-PLA bags (Figure 1). In agreement, steady state CO_2 and O_2 concentrations of 5-7 kPa and 9-6 kPa, respectively, have been shown to preserve the external color of celery (Gómez and Artés,

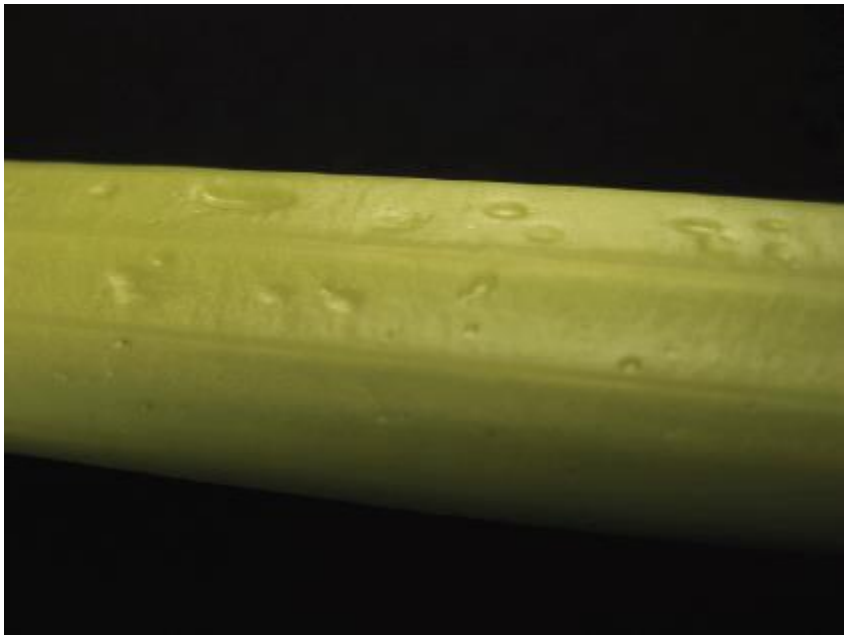
2005). Among headspace gas compositions, the CO_2-PLA bags maintained the green color of celery better than the other treatments ($P < 0.05$) up to d 14, after which all samples were visibly decayed. In contrast, celery sticks packaged in O_2-PLA showed a more intense yellowing compared to the other treatments ($P < 0.05$). In addition, celery sticks packaged in the O_2-PLA bags developed numerous small pits on the surface that became increasingly evident over time (Figure 2). The pitting development could be due to the high oxygen, but CO_2 accumulation could be also affecting, so further research needs to be carried out to clarify the origin of this disease. Surface lightness was affected by in-package gas composition, but not the packaging material with celery sticks packaged in CO_2-PLA bags exhibiting the lowest L* value during the first week of storage ($P < 0.05$) (Table 4).

Table 4. Lightness (L*) and hue (hº) values for surface and cut ends of the celery sticks packaged in different materials and initial gas compositions during storage at 7 ºC.

Analysis location	Packaging code	Storage time (days) 0		3		7		14		21	
		L*	**hº**	**L***	**hº**	**L***	**hº**	**L***	**hº**	**L***	**hº**
Surface	Open Bags	57.8±4.9	118.2±1.4	58.0±6.9 a*	117.5±1.9 ^{ab}	57.5±4.7 ^{a}	118.1±1.2 ^{b}	59.7±5.3 ^{ns}	117.4±2.3 ^{ab}	60.6±5.4 ^{ns}	116.9±2.4 ^{b}
	A-PP/PE	57.8±4.9	118.2±1.4	60.6±5.1 ^{b}	117.0±2.6 ^{a}	59.4±6.2 ^{ab}	117.8±1.4 ^{b}	60.5±5.6 ^{ns}	117.4±3.2 ^{ab}	60.2±4.8 ^{ns}	116.1±3.2 ^{a}
	A-PLA	57.8±4.9	118.2±1.4	59.0±6.1 ^{ab}	117.8±1.4 ^{bc}	59.3±5.0 ^{ab}	118.0±1.4 ^{b}	60.8±5.9 ^{ns}	117.7±2.2 ^{b}	60.5±5.0 ^{ns}	117.3±2.5 ^{b}
	O_2-PLA	57.8±4.9	118.2±1.4	59.1±5.3 ^{ab}	117.9±1.5 ^{bc}	60.9±5.7 ^{b}	117.3±1.7 ^{a}	60.6±5.7 ^{ns}	116.8±2.1 ^{a}	60.6±4.9 ^{ns}	115.7±2.9 ^{a}
	CO_2-PLA	57.8±4.9	118.2±1.4	57.8±5.9 ^{a}	118.9±1.9 ^{d}	58.8±5.7 ^{a}	118.8±1.4 ^{c}	59.9±6.3 ^{ns}	118.4±2.0 ^{c}	60.0±6.3 ^{ns}	117.3±2.1 ^{b}
	N_2-PLA	57.8±4.9	118.2±1.4	58.7±6.3 ^{ab}	118.3±1.4 ^{c}	59.0±5.9 ^{a}	118.3±1.7 ^{b}	60.8±5.7 ^{ns}	117.2±2.9 ^{ab}	59.2±5.3 ^{ns}	117.4±1.9 ^{b}
Cut ends	Open Bags	56.3±4.9	115.8±1.5	60.4±5.3 ^{b}	111.3±4.0 ^{a}	63.8±4.7 ^{c}	111.1±4.4 ^{a}	63.7±4.5 ^{c}	110.0±4.1 ^{a}	64.88±5.5 ^{d}	106.0±4.7 ^{a}
	A-PP/PE	56.3±4.9	115.8±1.5	59.1±5.2 ^{ab}	113.0±2.6 ^{b}	59.4±5.6 ^{a}	111.9±3.8 ^{ab}	59.7±5.9 ^{a}	111.3±3.7 ^{b}	60.3±5.5 ^{a}	107.6±5.6 ^{b}
	A-PLA	56.3±4.9	115.8±1.5	59.1±6.2 ^{ab}	113.7±3.0 ^{b}	61.9±5.9 ^{b}	113.4±3.0 ^{c}	62.4±5.8 b	112.9±3.2 ^{c}	61.9±6.2 ^{abc}	111.7±3.2 ^{d}
	O_2-PLA	56.3±4.9	115.8±1.5	60.6±5.8 ^{b}	113.4±2.6 ^{b}	61.8±5.1 ^{b}	112.4±3.1 ^{bc}	62.4±5.9 ^{b}	110.5±3.0 ^{ab}	61.3±7.0 ^{ab}	110.0±4.3 ^{c}
	CO_2-PLA	56.3±4.9	115.8±1.5	60.6±5.6 ^{b}	115.5±2.7 ^{c}	61.5±5.7 ^{b}	115.5±2.5 ^{c}	61.7±5.9 ^{ab}	114.3±2.9 ^{d}	63.5±5.5 ^{cd}	112.9±3.4 ^{d}
	N_2-PLA	56.3±4.9	115.8±1.5	57.8±7.5 ^{a}	115.3±2.5 ^{c}	61.6±5.6 ^{b}	114.8±2.8 ^{c}	61.9±6.2 ^{ab}	113.6±3.0 ^{cd}	62.5±5.0 ^{bc}	112.4±3.2 ^{d}

*Each value represents the mean of three replicates ± standard deviation. Different letters indicate significant differences ($P < 0.05$) between treatments for each time point and analysis location.

Figure 2. Celery stick pitting from O2-PLA bags after 21 days of storage at 7 ^{o}C.

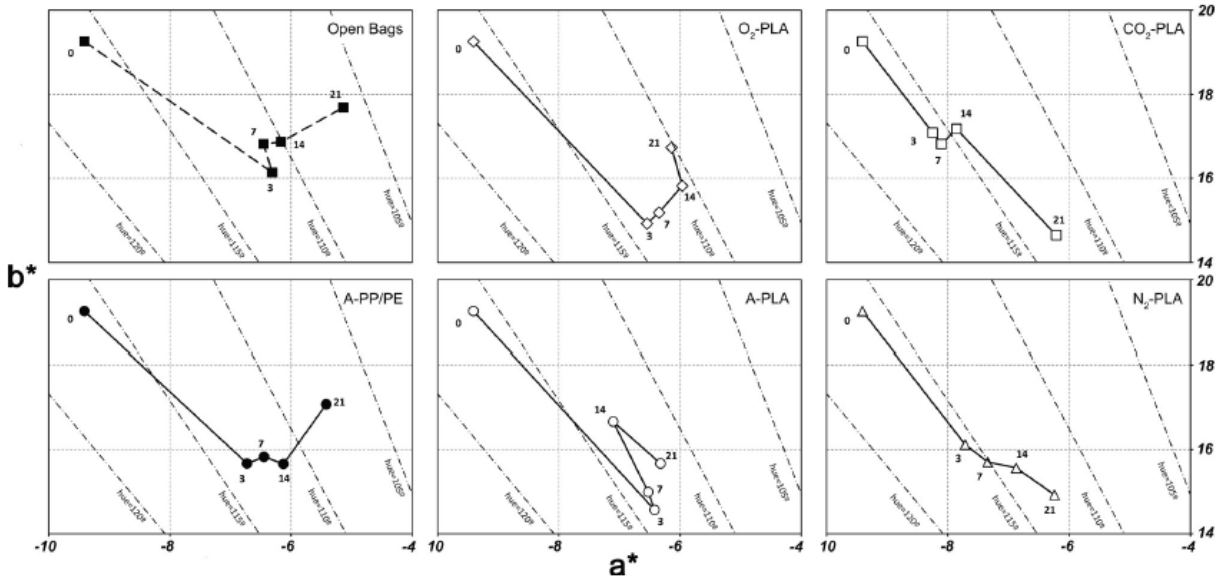


The color of cut celery stick ends was also affected by packaging material and initial gas composition (Table 4). Comparing the different materials, the cut celery stick ends in the A-PLA bags maintained a higher hue value (greener color) than those in A-PP/PE bags throughout storage. This was probably caused by the higher CO_2 content in the A-PLA bags. The effectiveness of low O_2 and high CO_2 levels in preserving the green color of the cut ends of fresh-cut celery in OPP bags at 4 ºC (6 kPa O_2 and 7 kPa CO_2 at steady state) was reported by Gómez and Artés (2005). However, initially high O_2 levels caused the degradation of the green color and promoted yellowing in the cut ends. In contrast, initially high O_2 levels (95 kPa) reduced discoloration of chicory endive (Jacxsens et al., 2001). CO_2-PLA and N_2-PLA maintained a higher hue during storage, frequently associated to the maintenance of a greener color. The L* value for the cut ends of celery rapidly increased during the first 7 d of storage and then stabilized thereafter (Table 4). Celery sticks stored at 10 ºC in passive modified atmospheres have reportedly exhibited similar behavior (Viña and Chaves, 2003) which is likely related to

increased hydration of the cut ends. Discoloration caused by reversible surface dehydration is common in fresh-cut produce including carrots (Tatsumi et al., 1991; Cisneros-Zevallos et al., 1995) and peaches (Gorny et al., 1998; González-Buesa et al., 2011). Although discoloration of cut ends due to lignin accumulation has been reported in many fresh-cut products, this does not seem to be the reason of the discoloration of the celery cut ends according to Viña and Chaves (2003). Polymerized phenol could be the cause of discoloration. Headspace gas composition had no effect on the L* value for celery cut ends, but packaging film did have an effect (Table 4). PP/PE bags better maintained lightness of the celery cut ends after 7 and 14 d of storage. Cut end lightness was associated with a lower level of dehydration compared to the PLA bags, with this higher L* due to the greater water vapor permeability of PLA (Table 4).

Figure 3 shows the change in the a* and b* color coordinates and of the hue angle in the cut ends of celery sticks during storage, providing additional information about the change in color. The color evolution of the cut ends of the celery sticks in the A-PP/PE bags was close to that of the cut ends of the celery sticks in the open bags, indicating that the CO_2 accumulated inside the A-PP/PE was not enough to avoid color degradation. Comparing the different initial gas compositions, the ends of the celery sticks in CO_2-PLA bags showed more stable a* and b* coordinates, especially during the first 14 d of storage, indicating a more well maintained color to the initial one. However, O_2-PLA and A-PLA showed a large change in the a* and b* values during the first 7 d, indicating major changes in the color of the celery cut ends. The N_2-PLA bags after 3 and 7 d maintained intermediate values, indicating a less severe color evolution.

Figure 3. Effect of initial gas composition and packaging material on the color (a*, b* and hue) evolution of the cut surfaces of celery sticks. Numbers (0, 3, 7, 14 and 21) indicate storage time in days at 7 ^{o}C.



Transversal dotted lines indicate different guide hue angles.

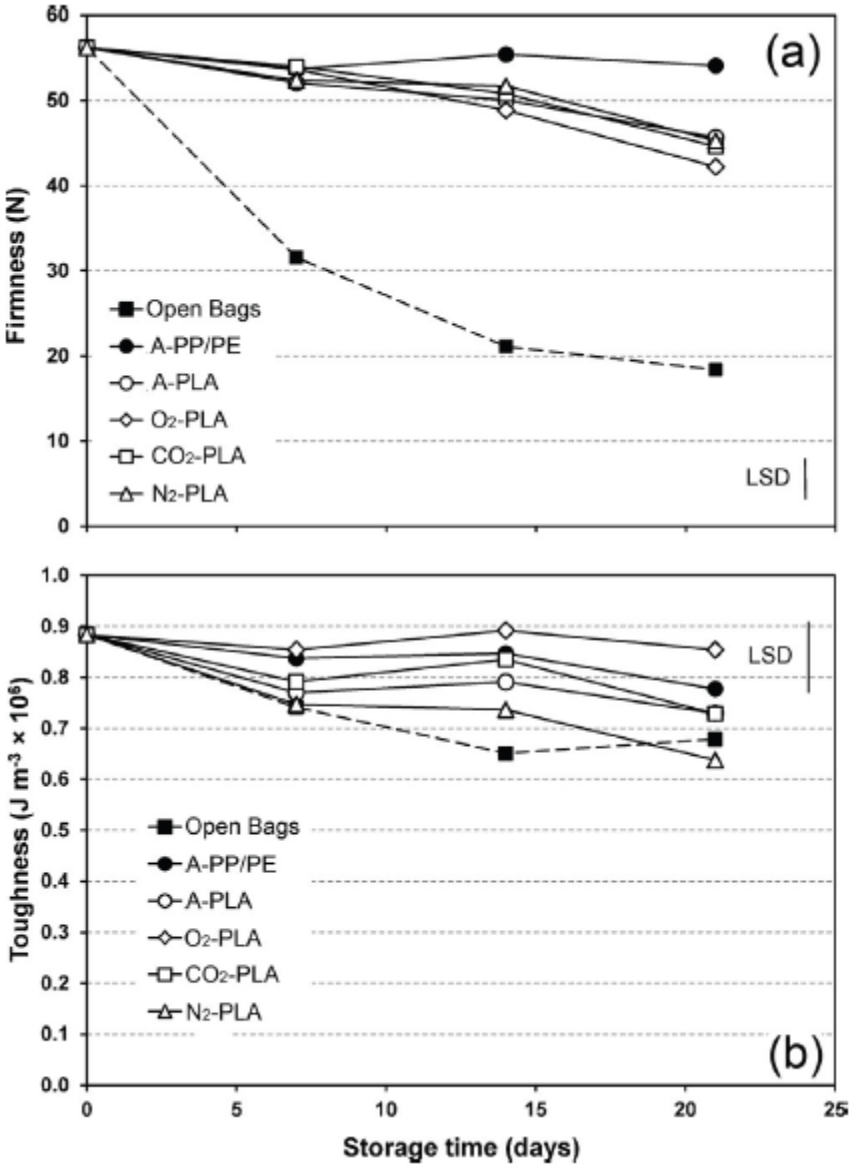
These results show that a high initial concentration of CO_2 in the headspace can help maintain the initial green color of the surface and cut ends of celery sticks while a high O_2 concentration promotes yellowing and other quality defects. While PLA can better maintain the green color of both cut and uncut celery, dehydration can become an issue.

3.2.5. Texture

Figure 4a shows the evolution of firmness or loss of turgor of unpackaged and packaged celery sticks during 21 days at 7 ºC, measured as the force at a displacement of 5 mm in the 3 bending point rig. Using open bags, celery stick firmness decreased from 56.2±10.6 N to 18.4±10.9 N during 21 d at 7 ºC. This change in texture is likely due to water loss and pectin degradation as suggested by Viña and Chaves (2003). In contrast, both PLA and PP/PE bags maintained celery firmness during 14 d of storage. At day 21, PLA-packaged celery softened due to the relatively high water vapor permeability of PLA while celery sticks packaged in PP/PE

maintained their turgor ($P < 0.05$) (Table 3). The different initial gas compositions did not affect the turgor of the celery sticks throughout the storage. This was due to the gas compositions having no effect on the weight loss of the celery sticks. Unlike firmness, the celery toughness was affected by the initial gas composition, but not the packaging material (Figure 4b). Celery sticks maintained a slightly tougher texture in high O_2 compared to other gas mixtures during storage ($P < 0.05$). This was most likely due to maintenance of the celery respiration rate at high O_2 levels, which has the potential to increase lignin production and hardening of the celery fibers. Viña and Chaves (2003) reported lignification of fibers and/or xylem vessels in fresh cut celery during storage in passive modified atmospheres (> 10 kPa O_2 and < 4 kPa CO_2) with similar results obtained for asparagus during exposure to 21 kPa O_2 (Everson et al., 1992). Another possible explanation is damage of tissues by the high O_2 exposure during storage.

Figure 4. Firmness (a) and toughness (b) of celery sticks packaged in different materials and initial gas compositions during storage at 7 ^{o}C.

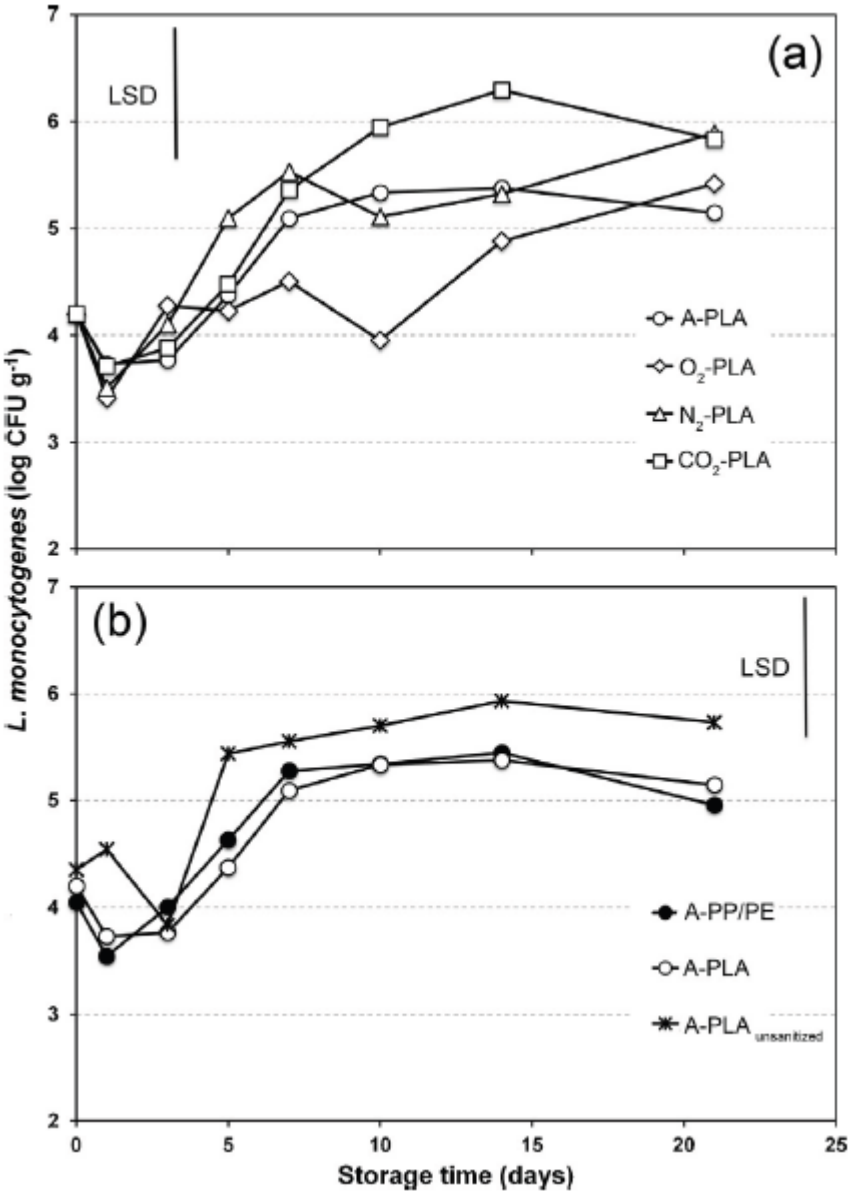


3.2.6. *Listeria monocytogenes*

Growth of *L. monocytogenes* on celery during 21 d of storage at 7 ºC was impacted by initial headspace gas composition (Figure 5a). While O_2-PLA bags showed no increase in *Listeria* populations during the first 10 d of storage ($P < 0.05$), CO_2-PLA, N_2-PLA, and A-PLA supported growth. Amanatidou et al. (1999) reported only a slight reduction in *Listeria* growth on agar plates that were exposed to 90 kPa O_2 at 8 ºC. In other work, *L. monocytogenes* growth

in fresh, processed, mixed salads (endive, curly endive, radicchio, lollo rosso and lollo bionda) was not affected by an initial oxygen level of 95 kPa during storage at 4 ºC (Allende et al., 2002). Suppression of *Listeria* growth is most likely only visible above 4 ºC. In fact, *Listeria* populations in fresh-cut celery stored at multiple temperatures in highly permeable bags and containers (< 2 kPa CO_2 and ≈ 18 kPa O_2 at steady state) decreased at 4 ºC due to the low temperature but not the gas composition (Vandamm et al., 2013).

Figure 5. *Listeria monocytogenes* populations on sanitized and unsanitized celery sticks packaged in different materials and initial gas compositions during storage at 7^{o} C ((a) compares initial headspace gas compositions and (b) compares materials as well as sanitation).



After 10 and 14 d of storage at 7 ºC, *Listeria* populations in the CO_2-PLA bags were slightly higher than those in the N_2-PLA and A-PLA bags, and significantly higher ($P < 0.05$) than those in the O_2-PLA bags (Figure 5). Enhanced growth of *Listeria* at higher CO_2 levels has been previously reported for other types of produce including cut chicory endive (Bennik et al., 1996), shredded cabbage (Kallander et al., 1991), and shredded lettuce (Francis and O'Beirne,

2001). In contrast, an initial in-package gas composition of 5 kPa O_2 + 5 kPa CO_2 suppressed *Listeria* growth in shredded iceberg lettuce (Carrasco et al., 2008). These observed differences in *Listeria* behavior in packaged fresh-cut produce stored at low temperatures can be partially explained by the type of produce, modified atmosphere, inherent microflora and *Listeria* strain variations (Caleb et al., 2013).

At day 21, no differences ($P > 0.05$) were found between the *Listeria* populations on celery for the different packaging methods, likely due to similarly low O_2 levels in all packages. The O_2-PLA bags were the only packages able to maintain the initial *Listeria* populations during the first 10 d of storage, but no longer, due to the shift to anaerobic conditions. This can be attributed to the lack of competing aerobic microflora that occurred when O_2 was depleted. According to Farber et al. (1998), decreased growth of *L. monocytogenes* on cabbage packaged using a highly-permeable film could be partially explained by the competing microflora such as lactic acid bacteria.

No differences ($P > 0.05$) were found between the *Listeria* populations on celery sticks packaged in different materials and between sanitized and unsanitized celery throughout storage (Figure 5b), even though the CO_2 level was higher and the O_2 level lower for the PLA bags.

4. Development of Novel Descriptive Sensory Methods to Evaluate the Quality of Fresh-cut Celery

4.1. Materials and methods

4.1.1. Sample preparation

Fresh celery bunches were purchased from a local distributor (Stan Setas Produce, Lansing, MI, USA), stored under refrigeration, and used within 1 day of delivery. All bunches were rinsed with ~4 °C tap water for 1 minute, before being manually cut into 10 cm sticks. The cut sticks were sanitized in 70 ppm chlorine in ~4 °C tap water for one minute and drained in a kitchen centrifuge. Before packaging, the celery sticks were carefully inspected and selected based on similar size and visual appearance.

A polylactic acid (PLA) film (44-µm thick) coated with high molecular weight PLA (EVLON EV-HS1, BI-AX International Inc., Wingham, ON, Canada) was used as the packaging material in this study. The O_2, CO_2 and water vapor permeabilities were determined in previous work (González et al., 2013) and are as follows: $30.34 \pm 9.07 \cdot 10^{-18}$ Kg m m^{-2} sec^{-1} Pa^{-1}, $5.67 \pm 1.17 \cdot 10^{-18}$ Kg m m^{-2} sec^{-1} Pa^{-1}, and $21.86 \pm 3.22 \cdot 10^{-15}$ Kg m m^{-2} sec^{-1} Pa^{-1}, respectively. Packaging film was formed into 20 x 15-cm bags using an impulse sealer (Ceratek, Sencorp Systems Inc., Hyannis, MA, USA). Approximately 190 grams of selected fresh-cut celery was packaged into each PLA pouch.

PLA pouches packaged with fresh-cut celery were stored at 5°C for 21 days. Samples were evaluated by a trained descriptive sensory initially (Day 0) and after 7, 14, and 21 days as explained below. Samples were prepared in triplicate (from 3 different bags) at each analysis day.

4.1.2. Panelist Selection

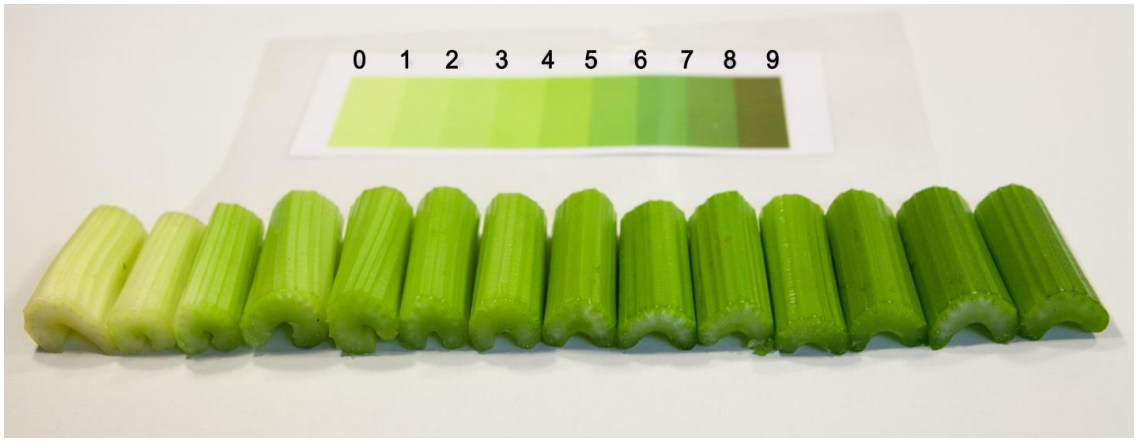
Panelists to be trained for quantitative descriptive sensory method (QDA) were selected based on their ability to discriminate. Twenty students and faculty members from Michigan State University (East Lansing, Michigan) were contacted based on their involvement with fruit and vegetable research or previous trained sensory panel experience. A preliminary screening assessment using triangle tests was administered to potential panelists, to evaluate their ability to discriminate between celery colors and textures. From the screening test results, nine panelists were selected to be trained to evaluate fresh-cut celery throughout storage.

4.1.3. Development of novel descriptive sensory methods and training of panelists

4.1.3.1. Standardized color scale

A standardized color scale for the assessment of color changes in fresh-cut celery was developed from PANTONE® colors. The scale was based on a 9-point scale (1-yellow/light green to 9-dark green) using nine PANTONE® colors. The standard PANTONE® colors from light to dark green used for the scale were 587, 586, 585, 584, 381, 382, 583, 378, and 581. The complete color scale is featured in Figure 6 along with a typical range of celery sticks from one bunch. Panelists were trained to use the scale under consistent fluorescent lighting conditions in individual evaluation booths. The upper/outer side of the fresh-cut celery sticks were used for color evaluation.

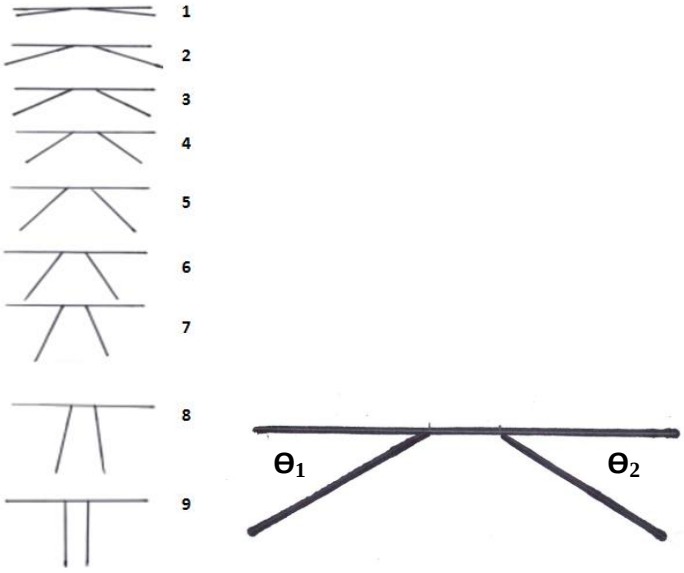
Figure 6. Pantone® celery color scale



4.1.3.2. Flexibility texture method

A new 9-point QDA reference scale (1-very crisp to 9-very flexible) was developed to assess the flexibility of fresh-cut celery. The scale is diagramed in Figure 7a and represents product breakage angles from 10° to 90°. The references were developed to show breakage angle increments at every ten degrees. Panelists were trained to press celery sticks (with the outer edge facing the panelist) along their entire length onto an ink pad, transfer them to an anchored piece of paper, and bend them with constant pressure until they snap. The inked side of the celery stick remained in contact with the paper while bending. The angle created between the initial ink impression and the impression at the point where the celery stick broke was compared to the reference scale. Figure 7b diagrams the two angles created by the ink impressions when bending celery, signified as Θ_1 and Θ_2. The flexibility of the celery was evaluated by taking the average of the two angles.

Figure 7 a) Bending Scale used as a reference to compare with the ink impressions created from bending celery sticks (1-very crisp to 9-very flexible) and b) angles (Θ_1 and Θ_2) of the ink impressions created from bending celery sticks.



4.1.3.3. Hardness sensory method

A universal 15-point spectrum scale was used to evaluate the hardness of fresh-cut celery (Meilgaard, 1999). The scale was slightly modified to fit within a 9-point range for celery. Within the 15-point scale, celery was determined to fit within the range of 7 to 12 (5-very soft celery to 14-very hard celery). Standard references for hardness included one whole pitted green olive (7), one whole roasted peanut (9), ½ inch cube of carrot (11), and one whole roasted almond (12). Panelists were trained to compress reference and fresh-cut celery samples between their molars and compare their hardness (the force it takes to completely bite through the sample) throughout storage.

4.1.4. Statistical analysis

Analysis of variance and the Tukey-Kramer test were used to determine any significant differences at a confidence level of 95% ($P \leq 0.05$). Statistical analysis was performed with SAS version 9.3 software (SAS Institute, Cary, N.C., U.S.A.)

4.2. Results and discussion

4.2.1. Texture

Firmness relates to the softening of the plant tissue and the strength and thickness of the cell wall are major contributors (Toivonen and Brummell, 2008). Therefore, the less firm the product is, the more flexible the product will become. As celery ages, celery firmness decreases and subsequently, celery flexibility increases. A new QDA was developed to assess changes in celery flexibility. For that, trained panelists were asked to bend celery sticks and compare their results to a reference scale. The standard bending scale represents the firmness of the celery sticks by relating their flexibility to the angle at which the product breaks when it has been deformed by bending. The new QDA method was compared against a universal spectrum method used for measuring hardness. Like a change in celery flexibility, a change in the hardness of celery indicates that the firmness of the celery is changing. The results for bending and hardness evaluations by 9 trained panelists over a 3 week storage period can be seen in Table 5. Average scores by panelists are shown out of a 9 point scale for each attribute ± standard deviation.

No significant differences were found for the flexibility of celery sticks between evaluation dates by the trained panel (Table 5). Therefore, the PLA bag seems to be able to retain enough moisture from leaving the bag that results in a maintained firmness of the celery sticks over a storage period of 3 weeks at 5°C. No significant differences in the sensory assessment of hardness were observed in celery sticks that had been stored in PLA bags either (Table 5). The lack of observable changes in sensory hardness could be attributed to the stability of the celery fibers. Therefore, results from hardness evaluated by the spectrum method and from flexibility

evaluated by the developed QDA method are in agreement and show no differences in the firmness of the packaged celery sticks over a storage period of 3 weeks at 5°C.

4.2.2. Color

The Pantone® color scale (Figure 6) was used to indicate the light to dark green color spectrum of celery and observe any sensory color changes. Sensory tests performed showed that the celery studied did not significantly differ in green color over time (Table 5). The effectiveness of low O_2 and high CO_2 levels in preserving the green color of fresh-cut celery at 4 ºC (6% O_2 and 7% CO_2 at steady state) was reported also by Gómez and Artés (2005).

Table 5. Quantitative descriptive sensory analysis of the texture and color of fresh cut celery stored at 5 °C for 21 days.

Sensory Method	**Time (Days)**			
	0	7	14	21
Bending	$3.1 \pm 0.8^{a*}$	3.1 ± 0.6^{a}	3.2 ± 0.8^{a}	3.8 ± 0.9^{a}
Hardness	8.7 ± 0.7^{a}	8.7 ± 0.6^{a}	8.7 ± 0.6^{a}	8.8 ± 0.8^{a}
Surface Color	7.4 ± 0.4^{a}	7.0 ± 0.9^{a}	6.7 ± 0.7^{a}	7.2 ± 0.9^{a}

* Each value represents the mean of three replicates ± standard deviation. Means sharing the same lowercase letter indicate no significant difference ($P < 0.05$) between time points for each attribute.

These results indicate that for the storage conditions evaluated, celery sticks were able to maintain their sensorial properties for 3 weeks. No differences were observed for flexibility (bending method), hardness, or color, indicating that PLA is a viable packaging option for fresh-cut celery sticks stored at 5 °C for up to 3 weeks. It is worth noting that these results to not take into consideration off-flavor development or consumer liking of the packaged celery sticks.

5. Interactions Between Sanitizers and Packaging Gas Compositions and their Effects on the Growth of Spoilage Microorganisms and *Salmonella* on Fresh-cut Onions (*Allium cepa* L.)

5.1. Materials and methods

5.1.1. Onion dicing

Spanish yellow onions (*Allium cepa* L.) were purchased from a local distributor (Stan Setas Produce, Lansing, MI, USA), stored under refrigeration, and used within 1 day of delivery. All onions were visually inspected, had their ends and outer layers manually removed and finally were diced using an Urschel mechanical dicer model HA (Valparaiso, IN, USA). The same procedures were repeated using three different batches of onions to obtain three replicates.

5.1.2. Preparation of inoculum and inoculation of the diced onions

Stock cultures of one avirulent strain *Salmonella enterica* subspecies enterica serovar Typhimurium (LT2 , obtained from D. Michelle Danyluk, University of Florida, Gainesville, FL, USA) were maintained at -80 °C and subcultured twice (24 h/37 °C) in 9 mL of trypticase soy broth (Difco, Becton Dickinson, Sparks, MD, USA) containing 6 g/L yeast extract (Difco, Becton Dickinson, Sparks, MD, USA). The culture was diluted in tap water (~2 ºC) to contain 5 log CFU/ml and then the diced onions (~ 4 ºC) were immersed for 2 min. This led to diced onions with 3.87 ± 0.05 log CFU/g.

5.1.3. Sanitation of the diced onions

Approximately 23 kg per replicate of both *Salmonella*-inoculated and un-inoculated diced onions were sanitized by immersion for 2 minutes in 60 L at ~2 ºC of one of the following commercial sanitizers: 0.08 g/L sodium hypochlorite at pH 6.5 (XY-12, Ecolab, St. Paul, MN, USA), 0.08 g/L peroxyacetic acid at pH 5.9 (Tsunami 100, Ecolab, St. Paul, MN, USA), or 0.002 g/L liquid chlorine dioxide (CDG Environmental, Bethlehem, PA, USA). Liquid chlorine

dioxide was used as a control because it is currently used to sanitize packaged diced onions according to industry contacts. Concentrations of active sodium hypochlorite and peroxyacetic acid were measured using a chlorine or peracid/peroxide test kit #321 and #311 (Ecolab, St. Paul, MN, USA), respectively, while chlorine dioxide concentration was measured using chlorine dioxide test strips (InstaTest UX-99532-34, Cole-Parmer, Vernon Hills, IL, USA). The sodium hypochlorite wash was acidified according to manufacturer's instructions by adding a 100 g/L citric acid solution and monitoring the pH until stable. After sanitation, the excess sanitizing wash was spun off of the diced onions using a SD50-LT spin drier (Heinzen Manufacturing Intl., Gilroy, CA, USA).

5.1.4. Packaging of the diced onions

The centrifugally dried *Salmonella*-inoculated and un-inoculated diced onions were weighed out into 100 g batches for each individual package. Two different packaging systems were used: snap-fit containers and bags. The bags with diced onions that were to be filled with an active modified atmosphere were placed into a sterilized glovebox chamber (Labconco 50004 Fiberglass Glove Box, Kansas City, MO, USA). The chamber was closed and then flushed with the appropriate atmosphere (99 kPa O_2 + 1 kPa N_2 or 15 kPa CO_2 + 5 kPa O_2 + 80 kPa N_2). Finally, the bags were sealed using an impulse sealer located inside the chamber to obtain active modified atmosphere packages. Passive modified atmosphere packages were obtained by sealing the remaining bags under air. Snap-fit containers were simply filled with 100 g batches and the lids were fastened on before storage. Packaging was conducted under sterile conditions at ~4 ºC. All packages were stored at 7 ºC until the appropriate analysis day.

5.1.4.1. Packaging system characterization

Snap-fit containers made of polyethylene terephthalate (PET) (Clear Lam, Elk Grove Village, IL, USA) had approximate internal dimensions of 9.5 × 9.5 × 2.5-cm, with a total volume of 210 mL, an external surface of 270 cm^2, and an average thickness of 330 µm. These containers were chosen to mimic what industry is currently using for diced packaged onions based on information provided from industry contacts. Bags were made of polylactic acid (PLA) film (EVLON EV-HS1, BI-AX International Inc., Wingham, ON, Canada) and were formed using an impulse sealer (Ceratek, Sencorp Systems Inc., Hyannis, MA, USA). PLA was chosen as the packaging material because of its consumer desired sustainability properties (Koutsimanis et al., 2012), it has better thermal processibility than other biopolymers (Rasal et al., 2010), and can be processed on standard converting equipment with minimal modifications (Lim et al., 2008). The approximate internal bag dimensions were 11 × 12.5-cm, resulting in a total area of 275 cm^2.

O_2, CO_2, water vapor, and ethanol transmission rates of the whole packages (snap-fit container and sealed bag) were determined. For the snap-fit containers, due to the presence of a non-hermetically sealed lid, the whole package transmission rate for O_2 and CO_2 was measured using a static method, adapting the procedure described by González et al. (2008) to a whole package. Water vapor and ethanol transmission rates in the snap-fit containers were measured using a gravimetric method. 12 mL of water or ethanol was placed on the bottom of the packages, the lids were then snapped and their weight losses were measured over time using a precision balance (OHAUS, model Discovery DV314C, Florham Park, NJ, USA). All testing was conducted in triplicate and the results were expressed as kg/s·package.

O_2, CO_2, water vapor, and ethanol transmission rates of the PLA bags were estimated by using the permeability values of the PLA film to these gases and vapors previously calculated by this research team (30.34±9.07 and 5.67±1.17 × 10^{-18} kg·m/m^2·s·Pa for CO_2 and O_2, respectively, 21.86±3.22 × 10^{-15} kg·m/m^2·s·Pa for water vapor, and 20.01±5.76 × 10^{-18} kg·m/m^2·s·Pa for ethanol (González-Buesa et al., 2014)), the average thickness of the PLA film, the total area of the bag, and the pressure difference of gases and vapors. For O_2 and CO_2, the pressure difference was 101,350 Pa, and for water vapor and ethanol, the pressure difference was 1,408 Pa and 7,821 Pa, respectively. The results were expressed as kg/s·package.

5.1.5. Analyses

5.1.5.1. Microbiological analyses

After 0, 3, 7, 10, and 14 days of storage at 7 °C, 25 g of *Salmonella*-inoculated or un-inoculated diced onions from each package were diluted 1:1 in Difco Neutralizing Buffer (Difco, Becton Dickinson, Sparks, MD, USA) and homogenized in a stomacher (Stomacher 400 Circulator, Seward, Worthington, UK) at 260 rpm for 1 min. Un-inoculated samples were appropriately diluted in sterile 1 g/L phosphate buffer solution and plated on either trypticase soy agar + yeast extract (Difco, Becton Dickinson, Sparks, MD, USA) for quantification of mesophilic bacteria after 24 h of incubation at 37 °C, or potato dextrose agar (PDA) (Neogen Corporation, Lansing, MI, USA) acidified to pH 3.5 with a 100 g/L tartaric acid solution for quantification of yeasts and molds after 48 h at 23 °C (following the Neogen Accumedia method for PDA #7149). Inoculated samples were also serially diluted but then plated on the selective and differential media brilliant green agar (Neogen Corporation, Lansing, MI, USA) for quantification of *S.* Typhimurium after 24 h of incubation at 37 °C.

5.1.5.2. Physico-chemical analyses

5.1.5.2.1. In-package atmosphere

In-package O_2, N_2, and CO_2 levels were determined using a gas chromatograph (Thermo Scientific Trace GC Ultra, Thermo Electron S.p.A., Rodano, Italy) equipped with a thermal conductivity detector and a Carboxen 1010 Plot capillary column, 30 m in length with a film thickness of 30 µm, and an internal diameter of 0.53 mm (Supelco, Bellefonte, PA, USA). Helium was used as a carrier gas (3 mL min^{-1}). Using a syringe (SGE, Austin, TX, USA), a 100-µL headspace sample was removed through a silicone septum that was attached to each package on the appropriate headspace sampling day. The initial gas compositions of all the packages were determined to verify correct flushing by the glovebox. Subsequently, O_2, N_2, and CO_2 levels were monitored on days 2, 6, 9, and 13 of storage at 7 °C. Snap-fit packages were also monitored throughout storage for headspace gas concentrations. These results are not shown because the in-package O_2, N_2, and CO_2 levels were the same as those in atmospheric air.

In-package ethanol concentrations were determined after 7, 10, and 14 days of storage at 7 °C by injecting 1 mL of headspace gas into a gas chromatograph equipped with a flame ionization detector (Hach Carle Series 100 AGC, Loveland, CO, USA) fitted with a 2 m long, 2 mm internal diameter stainless steel column packed with Chromosorb OV-103, 60/80 mesh (Altech Associates Inc., Deerfield, IL, USA).

5.1.5.2.2. Color

Diced onion color was determined after 0, 3, 7, 10, and 14 days of storage at 7 °C using a Minolta CR300 colorimeter (Osaka, Japan) calibrated with a standard white plate (Y=93.84; x=0.3132; y=0.3191) and set with a C illuminant, 2º observer. Four samples of diced onions were taken from each package and were then placed into glass sample cups (#04720900 Hunter

Lab, Reston, VA USA). Each sample cup was measured in three different places and L* and b* values recorded. The average value of the twelve measurements was used to represent package L* and b* values for each replicate.

5.1.5.2.3. Weight Loss

The weight loss of the diced onions was determined after 3, 7, 10, and 14 days of storage at 7 °C for all treatments by weighing the entire package over time and using the formula shown below, with the results expressed as % weight loss.

$$\left[\frac{(Wi - Wf)}{Wi - Wp}\right] * 100$$

Where: Wi is the initial weight of the diced onions plus the weight of the package, Wf is the final weight of the diced onions plus the weight of the package, and Wp is the weight of the package.

5.1.5.2.4. pH

The pH of the diced onions was measured on day 0, 3, 7, 10, and 14 of storage at 7 °C using an Omega PHB-212 pH meter (OMEGA Engineering, Inc., Stamford, CT, USA), calibrated everyday of testing with buffer solutions at pH 4 and 7. Approximately 10 g of diced onions from each package were placed into sterile 50 mL centrifuge tubes (Corning, Tewksbury, Massachusetts, USA), mixed with 10 mL of distilled water, and homogenized using a PT-2000 polytron (Kinematica, Lucerne, Switzerland) for 30 seconds. The pH of the resulting solution was measured twice and the average value was used to represent package pH. Between each sample the pH meter probe was rinsed with distilled water and patted dry.

5.1.5.3. Sensory Analyses

5.1.5.3.1. Descriptive sensory analysis

Descriptive sensory analysis was performed to determine the acceptable storage period for diced onions packaged with the best atmosphere/sanitizer combination for maintenance of

physico-chemical and microbiological quality when compared with freshly packaged diced onions. Panelists experienced with fresh produce quality and previous experience on a trained sensory panel were recruited and screened based on their ability to discriminate between samples of freshly diced onions and diced onions stored at 23 ºC for 12 hours using a triangle discrimination test. The eight panelists selected were then trained to use 5-point intensity scales prior to sample evaluation. Two samples of packaged diced onions were provided to the panelists for evaluation on days 0, 3, 7, 10, and 14 of storage at 7 °C . The two samples were 1) the best packaging treatment as predetermined in the physico-chemical and microbiological results, and 2) packages containing freshly diced onions (less than 12 h). Panelists were also shown fresh-diced onions to remind them of the desired characteristics for fresh onions each testing day. Attributes of color, "off-odor", and overall appearance were rated using 5-point intensity scales. Color and overall appearance were evaluated from the outside of the package for both samples first, followed by off-odor. To determine off-odor, panelists were given a small needle to poke a hole in the bag so as to not overwhelm their olfactory sense by opening the entire bag. Finally, the panelists were asked to determine if the packaged onions were acceptable for purchase at the grocery store based on being trained as to what was an acceptable level of quality. Samples for sensory evaluation were prepared under the same conditions used for the other portions (microbiological and physico-chemical) of this study, including the use of the commercial dicer to best mimic industry conditions. However, since the commercial dicer was previously used with inoculated samples, it was not considered safe to ask panelists to consume the diced onions. Because of this, panelists were restricted to the attributes and chosen methods (i.e. closed packages) described above. The descriptive sensory training and analysis were held in the

sensory laboratory at Michigan State University, under controlled conditions (light, temperature, aroma) with each panelist performing the test at their own booth to minimize distractions.

5.1.5.3.2. Consumer acceptance sensory analysis

A consumer acceptance sensory analysis was performed to determine the acceptability of the packaged diced onions on day 14 by consumers. Eighty-five consumers of fresh onions were recruited and asked to evaluate the same two sample packages as the descriptive sensory analysis panelists evaluated. For both packages, panelists were provided a hedonic 9-point scale and asked to evaluate the acceptability of the onion aroma from outside the package, since this is how they would evaluate the package in the grocery store. They were also asked to rate their acceptance of the onion color and overall appearance of the packaged diced onions. Finally, they were asked if they would consider this product to be acceptable for purchase at the grocery store. In addition, panelists were asked about their fresh-cut produce and onion purchasing behavior and for feedback as to what would make them more likely to purchase fresh-cut onions if they were not already. Panelists were asked to evaluate the aforementioned attributes and not to open the packages for the same reasons as presented for the trained panel. The consumer acceptance sensory test was held in the sensory laboratory at Michigan State University, under controlled conditions (light, temperature, aroma) with each panelist performing the test at their own booth to minimize distractions.

5.1.5.4. Statistical analysis

Both microbiological and physico-chemical analyses were conducted on three replicates on each analysis day, and data were expressed as means ± standard deviation. Each of the three replicates consisted of three packages: one was used for microbial analysis and the other two were used for physico-chemical analyses. Three data points were obtained for *Salmonella*,

mesophilic bacteria, yeast/molds, and pH while six data points were obtained for O_2, N_2, CO_2, ethanol, and weight loss on each analysis day. A total of 36 data points were obtained for color on each analysis day. Differences caused by time, atmosphere, and sanitizer and their interactions for all the evaluated parameters were determined through two-way analysis of variance (ANOVA) performed using the statistical software package IBM SPSS version 19 (IBM Corporation Software Group, Somers, NY, USA) and confirmed with the GLIMMIX procedure of SAS (SAS Inst. Cary, NY). Additionally, one-way ANOVA was performed using the statistical software package IBM SPSS version 19 to show differences caused by time, atmosphere, and sanitizer for weight loss, ethanol and pH at each analysis day. Tukey's test at a significance level $P < 0.05$ was used for both one-way and two-way ANOVA. The means of the microbiological, color (L* and b*), and headspace analyses were also compared with the least significant difference (LSD) test ($P < 0.05$).

5.1.6. Treatment labels

As previously described, all onions were sanitized with one of three sanitizers and packaged in PLA bags containing either active or passive modified atmosphere or in PET snap-fit containers which always contained atmospheric air. For simplicity, throughout this paper the atmosphere/sanitizer combinations are presented as codes comprised of the abbreviation of the initial in-package atmosphere, followed by the abbreviation of the sanitizer type used. Table 6 shows the abbreviations that comprise the codes (i.e., PA O2 for onions sanitized with peroxyacetic acid and packaged in 99 kPa O_2).

Table 6. Packaging and sanitizer abbreviations that comprise the codes used in this paper.

Sanitizer	Sanitizer Abbreviation	Packaging Abbreviation	Initial Atmosphere/ Packaging Design
Sodium hypochlorite	Cl	O2	99 kPa O_2 + 1 kPa N_2 / Bag
Peroxyacetic acid	PA	CO2	15 kPa CO_2 + 5 kPa O_2 + 1 kPa N_2 / Bag
Chlorine dioxide (liq.)	ClO2	Air	21 kPa O_2 + 79 kPa N_2 / Bag
		SF	21 kPa O_2 + 79 kPa N_2 / Snap-fit

5.2. Results and discussion

5.2.1. Packaging system characterization

O_2, CO_2, WV, and ethanol transmission rates of the PLA bag and the PET snap-fit container are shown in Table 7. The O_2, CO_2, and ethanol transmission rates of the PET snap-fit container are higher than those of the PLA bag. The greater gas exchange in the PET snap-fit container is due to the significant diffusion of O_2, CO_2, and ethanol through air due to its non-hermetic closure. In fact, the diffusion coefficient of O_2 and CO_2 through air was obtained to be approximately 450 and 62 million times, respectively, greater than through the PET sheet when using the approach of Yasuda (1975) to make such comparisons possible, and the diffusion coefficient of O_2 and CO_2 in air provided by Mannaperuma et al. (1999) and Massman (1998), respectively. In contrast, the WV transmission rate of the PET container was lower than that of the PLA bag. This resulted from the weaker barrier to WV of PLA compared to PET (Almenar and Auras, 2010), the reduced thickness of the PLA film compared to the PET sheet, and the lower water vapor exchange through the PET container closure than through the PET container wall. Based on these results, the PET snap-fit container behaves similar to a microperforated package, where microperforations have a greater impact on O_2 content than on WV content inside package (Fishman et al., 1996).

Table 7. Transmission rates (TR) of gases and vapors in the two packaging systems used in this study.

Packaging system	**Whole package TR** *(×10^{-10} kg/s·package)*			
	O_2	***CO_2***	***WV***	***Ethanol***
PET container[1]	1402 ± 91	1591 ± 117	45 ± 2	81 ± 29
PLA bag[2]	3.6 ± 0.7	19 ± 6	192 ± 28	1.0 ± 0.3

[1] Whole package TR values calculated in this study.

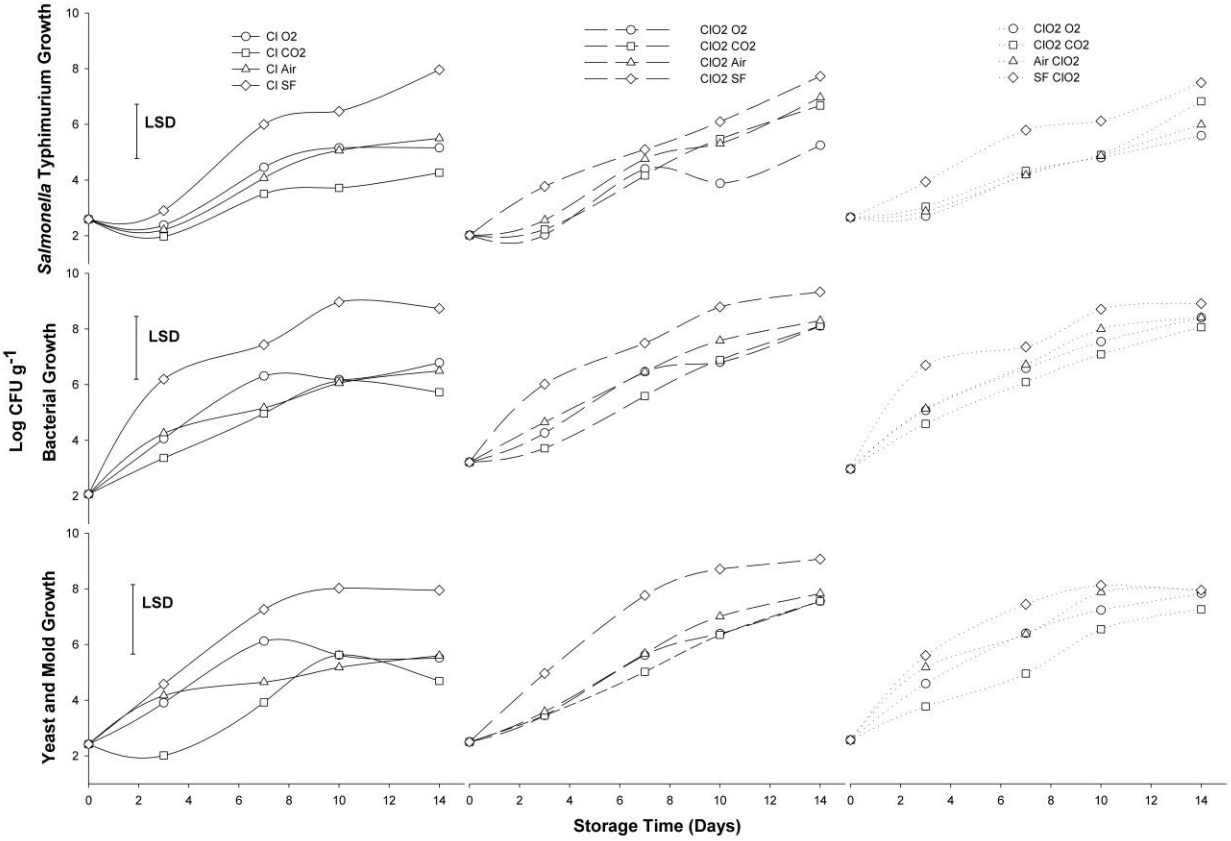
[2] Whole package TR values estimated from film permeability values in González-Buesa et al. (2014).

5.2.2. Effect of atmosphere/sanitizer combinations on microbial growth in diced onions

5.2.2.1. *Salmonella* Typhimurium

Figures 1 and 2 show the growth of *S.* Typhimurium on diced onions under different atmosphere/sanitizer combinations throughout two weeks of storage at 7 °C. Interactions between atmosphere and time (P = 0.003), sanitizer and time (P = 0.040), and atmosphere and sanitizer (P = 0.003) affected the growth of *S.* Typhimurium on packaged diced onions (Table 8). None of the studied atmosphere and sanitizer combinations were able to completely stop the growth of *S.* Typhimurium throughout time (P< 0.05), but some combinations were able to reduce growth compared to others. The snap-fit packages had the diced onions with the most *S.* Typhimurium growth (P < 0.05) throughout storage regardless of the type of sanitizer (Figure 8). Diced onions sanitized with ClO2 contained significantly (P < 0.05) more *S.* Typhimurium than those sanitized with Cl throughout storage regardless of the in-package atmosphere (Figure 9).

Figure 8. Effect of sanitizer/atmosphere combinations on the growth of *Salmonella* Typhimurium, Total aerobic bacteria, and yeasts and molds on diced onions during 14 days of storage at 7 ^{o}C.



This indicates that the commercially used package (snap-fit container) and sanitizer (liquid chlorine dioxide) are not effective at preventing *S.* Typhimurium growth in the instance of contamination. However, an in-package atmosphere other than air can reduce *S.* Typhimurium growth on diced onions ($P < 0.05$). This is consistent with findings by other authors. Amanatidou et al. (1999) reported that the combined application of high O_2 and moderate CO_2 had an inhibitory effect on the growth of nonpathogenic *S.* Enteritidis. Martínez-Hernández et al. (2015) showed a bacteriostatic effect on *S.* Enteritidis in fresh-cut broccoli sanitized with PA and stored in either passive MAP or active MAP (90% O_2) at 5 ^{o}C. .

Interactions between atmospheres and sanitizers that affect *S.* Typhimurium growth on diced onions were observed as well, with diced onions of the Cl CO2 treatment having the

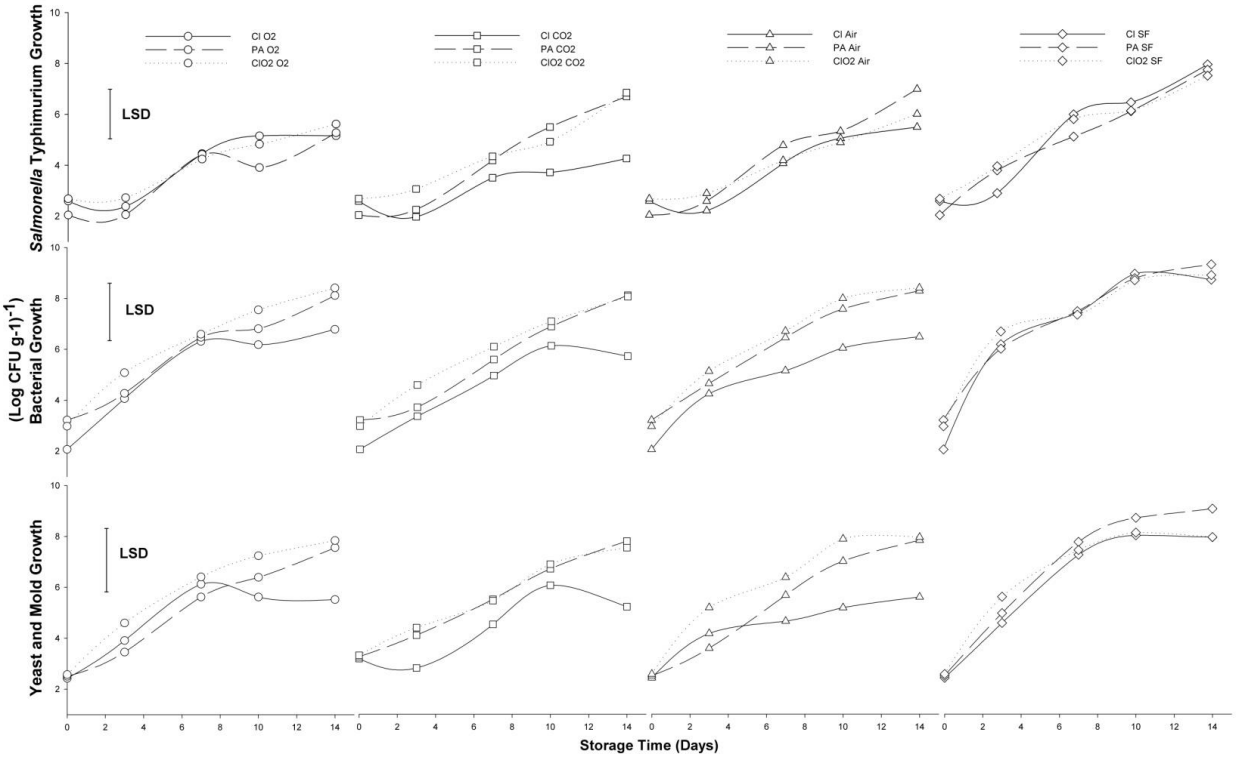
lowest *S.* Typhimurium populations throughout storage (about 1.5 log CFU/g of growth from day 0, compared to 2.5 - 5 log CFU/g of growth for all other treatments). Therefore, the combination of CO2 and Cl works to reduce *S.* Typhimurium growth in diced onions.

Table 8. P values resulting from Univariate ANOVA (SPSS) (P < 0.05 indicates effect of factor (single or combined (interaction)) on each parameter).

	Parameter									
Factor	*S.* Typhimurium	Bacteria	Yeasts&Molds	O_2	CO_2	Ethanol	L*	b*	Weight Loss	pH
Atmosphere	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
Sanitizer	0.019*	0.000*	0.000*	0.307	0.003*	0.530	0.804	0.462	0.000*	0.001*
Time	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
Atmosphere*Sanitizer	0.003*	0.212	0.075	0.066	0.061	0.520	0.203	0.873	0.105	0.011*
Atmosphere*Time	0.003*	0.021*	0.005*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
Sanitizer*Time	0.040*	0.271	0.005*	0.551	0.197	0.271	0.445	0.651	0.005*	0.247
Atmosphere*Sanitizer*Time	0.551	1.000	0.947	0.086	0.007*	0.996	0.778	0.949	0.199	0.476

* indicates significant (P < 0.05)

Figure 9. Effect of atmosphere/sanitizer combinations on the growth of *Salmonella* Typhimurium, Total aerobic bacteria, and yeasts and molds on diced onions during 14 days of storage at 7 ^{o}C.



5.2.2.2. Mesophilic bacteria

The effect of different atmosphere/sanitizer combinations on the growth of mesophilic bacteria on diced onions throughout two weeks of storage at 7 °C is illustrated in Figures 9 and 10. Mesophilic bacteria were affected by atmosphere and time (P = 0.021), sanitizer (P = 0.000), and time (P = 0.000) (Table 8). Snap-fit packages had diced onions with significantly higher (P < 0.05) mesophilic bacteria populations, 6 log CFU/g increase from day 0 to day 14, regardless of the type of sanitizer (Figure 8). Therefore, it seems that an in-package atmosphere other than air can reduce bacterial growth on diced onions. Howard et al. (1994) and Forney et al. (2012) reported the positive effect of CO_2 in reducing bacteria growth in packaged diced onions stored at low temperature. Blanchard et al. (1996) found that reduced O_2 accompanied with elevated

CO_2 controlled atmospheres reduced mesophilic and psychrotrophic growth in diced onions during 2 week storage at 4 °C. As for the effect of sanitizer on bacterial growth, diced onions sanitized with Cl had significantly lower ($P < 0.05$) mesophilic bacterial populations than those sanitized with PA or ClO2 regardless of the in-package atmosphere (Figure 9). Therefore, the commercially used package (snap-fit container) and sanitizer (liquid chlorine dioxide) are not the best choices for commercializing diced onions in terms of bacterial growth.

5.2.2.3. Yeasts and molds

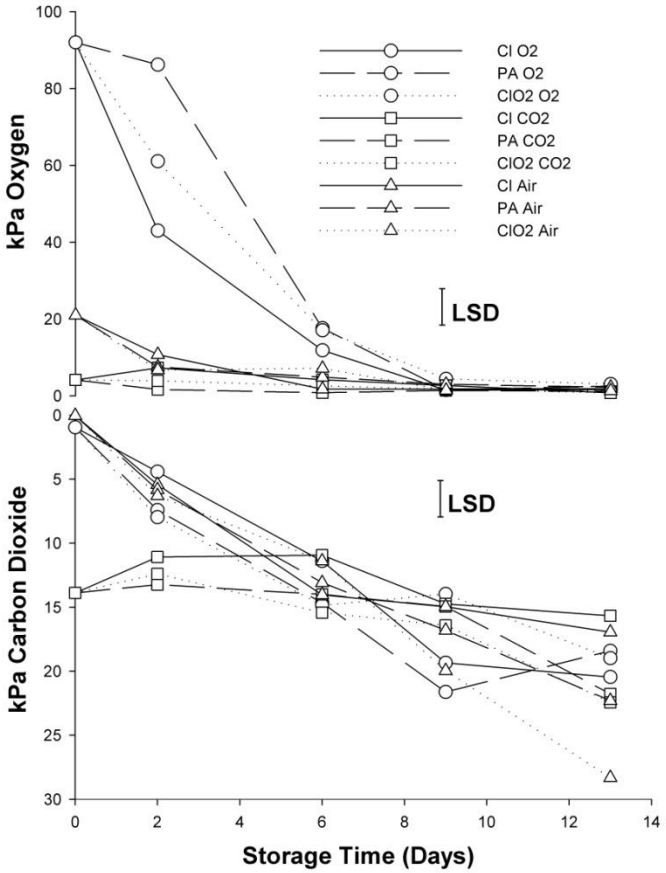
Yeast and mold population changes over time as affected by atmosphere/sanitizer combinations can be seen in Figures 1 and 2. Atmosphere, sanitizer, and time all had significant effects on yeast and mold growth ($P = 0.000$ for all three; Table 8). Snap-fit and CO2 in-package atmospheres had diced onions with the highest and the lowest yeast and mold populations ($P < 0.05$), respectively, compared to all other atmospheres regardless of sanitizer used (Figure 8). In contrast, Liu and Li found no significant differences between yeast populations on sliced onions packaged in 40 KPa CO_2 + 1KPa O_2 or air. Since the diced onions exposed initially to CO_2 had less yeast and mold populations than those exposed to the CO_2 build-up during storage (O2 and Air atmospheres; section 3.3.1), it can be concluded that the microbial quality in terms of yeasts and molds on diced onions can be improved using active MAP with high CO_2. Diced onions sanitized with Cl had significantly lower ($P < 0.05$) mold and yeast counts than those sanitized with either PA or ClO2 regardless of in-package atmosphere (Figure 9). These findings further prove that the current commercially used atmosphere/sanitizer combination is not the best option for packaging diced onions.

5.2.3. Effect of atmosphere/sanitizer combinations on physico-chemical changes in diced onions

5.2.3.1. In-package atmosphere

As expected, in-package atmosphere and time each had a significant effect (P = 0.000 for both; Table 8) on the O_2 levels inside the PLA bags, as seen in Figure 10. The bags with an in-package atmosphere CO2 reached O_2 equilibrium from the start (~1-2 kPa), while the bags with an in-package atmosphere Air equilibrated (~1.5-3 kPa) around day 7 of storage and those with an in-package atmosphere O2 did not reach O_2 equilibrium (~1.5-3 kPa) until approximately day 9. Sanitizer or interactions between the investigated factors did not have a significant effect on the O_2 levels inside the PLA bags throughout storage (Table 8). In contrast, sanitizer (P = 0.003) and 2-way (atmosphere and time; P = 0.000) and 3-way (atmosphere and sanitizer and time; P = 0.007) interactions affected the CO_2 levels inside the PLA bags (Table 8). CO_2 concentration steadily increased from 0 to ~ 20 kPa in the Air and O2 packages while it was maintained quite stable in the CO2 packages up to day 9 and then increased ~5 kPa. This lower accumulation of CO_2 in CO2 packages can be attributed to the faster reduction of O_2 occurring in these packages and/or the presence of high CO_2 which reduced the respiration rate of the diced onions. Similarly, Day (1989) observed a reduction in the respiration rate of cut onion under low O_2. Blanchard et al. (1996) suggested that both low O_2 and high CO_2 are necessary to reduce respiration in diced onions. The increase in CO_2 content of ~5 kPa after day 9 in the packages CO2 was only observed for the diced onions sanitized with PA or ClO2 (3-way interaction P = 0.007; Table 8). This could be attributed to the lower *S.* Typhimurium populations for this treatment (Figure 9), while the other treatments have greater microbial respiration attributing to their in-package CO_2 accumulation.

Figure 10. Effect of atmosphere/sanitizer combinations on in-package gas composition of diced onions during 14 days of storage at 7 ^{o}C.



Packages containing onions sanitized with Cl had significantly less ($P < 0.05$) CO_2 content than those sanitized with PA or ClO2, regardless of the in-package atmosphere. Gómez-López et al. (2008) observed an increased respiration rate in packaged RTU cabbage sanitized with ClO2 compared to unsanitized RTU cabbage and Martínez-Sánchez et al. (2006) observed an increase respiration rate in packaged rocket leaves sanitized with PA compared to lactic acid or acidified sodium chloride. In addition, diced onions sanitized with Cl had significantly lower ($P < 0.05$) mesophilic bacteria, mold and yeast counts than those sanitized with either PA or ClO2, regardless of in-package atmosphere (Figure 9). The greater microbial respiration in the packages with diced onions sanitized with either PA or ClO2 may have contributed to a higher in-package CO_2 buildup.

Headspace ethanol concentration (Table 9) was only significantly affected by in-package atmosphere throughout storage (interaction atmosphere and time; P = 0.000; Table 8). The packages containing diced onions exposed to the in-package atmosphere CO2 had the highest (P < 0.05) ethanol concentrations (80-100 ppm) while those in snap-fit packages and O2 had the lowest (P < 0.05) ethanol concentrations with no difference (P > 0.05) between them. Forney et al. (2012) attributed a rapid increase of ethanol content in packaged diced onions to low O_2 concentrations. This agrees with the presented results since the more rapid decrease of O_2 occurred in the packages with the in-package atmosphere CO2 regardless of sanitizer, and these were the packages containing the highest ethanol contents. The significantly greater (P < 0.05) in-package ethanol content for the CO2 packaged diced onions compared to diced onions packaged in Air, O2, and snap-fit containers could have also contributed to the lower yeast and mold populations of the CO2 packaged diced onions. Ethanol has been reported to reduce mold, yeast and bacteria growth and is most effective against mold (Almenar and Gartner, 2015). A difference in ethanol concentration between snap-fit containers and PLA bags was expected due to the faster diffusion of the ethanol through the non-sealed lid of the snap-fit container as shown in Table 7. However, this difference was not observed between snap-fit containers and PLA bags containing high O2 levels, which may indicate a lower production of ethanol in diced onions when exposed to high O2 levels compared to air.

Table 9. Effect of atmosphere/sanitizer combinations on the weight loss, in-package ethanol, and pH of packaged diced onions during 14 days of storage at 7 ^{o}C.

Parameter	Storage Time (Days)	Treatment											
		Cl O2	PA O2	ClO2 O2	Cl CO2	PA CO2	ClO2 CO2	Cl Air	PA Air	ClO2 Air	Cl SF	PA SF	ClO2 SF
Wt Loss	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
	3	0.26 ± 0.04 aB1	0.23 ± 0.04 aB1*	0.26 ± 0.05 aB1	0.28 ± 0.06 aB1	0.27 ± 0.03 aB1	0.29 ± 0.06 aB1	0.25 ± 0.05 aB1	0.25 ± 0.05 aB1	0.27 ± 0.04 aB1	0.03 ± 0.02 aA1	0.04 ± 0.01 aA1	0.06 ± 0.01 aA1
	7	1.15 ± 0.06 bB1	1.10 ± 0.23 bB1	1.18 ± 0.05 bB1	1.17 ± 0.08 bB1	1.17 ± 0.06 bB1	1.27 ± 0.09 bB1	1.17 ± 0.06 bB1	1.19 ± 0.22 bB1	1.15 ± 0.09 bB1	0.30 ± 0.01 bA2	0.23 ± 0.01 bA1	0.27 ± 0.02 bA2
	10	1.82 ± 0.08 cB1	1.75 ± 0.09 cC1	1.89 ± 0.12 cB1	1.88 ± 0.08 cB1	1.83 ± 0.04 cC1	1.91 ± 0.13 cB1	1.75 ± 0.08 cB2	1.63 ± 0.04 cB1	1.82 ± 0.05 cB2	0.57 ± 0.07 cA1	0.44 ± 0.04 cA1	0.46 ± 0.06 bA1
	14	2.82 ± 0.17 dB1	2.77 ± 0.27 dB1	2.92 ± 0.18 dB1	2.91 ± 0.15 dB1	2.69 ± 0.14 dB1	2.95 ± 0.23 dB1	2.82 ± 0.13 dB12	2.65 ± 0.13 dB1	2.94 ± 0.21 dB2	0.91 ± 0.06 dA1	0.83 ± 0.11 dA1	0.77 ± 0.15 cA1
Ethanol	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
	3	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
	7	8.59 ± 4.94 aAB1	6.07 ± 1.75 aA1	7.39 ± 4.42 aAB1	23.85 ± 15.76 aB1	15.5 ± 15.57 aA1	14.48 ± 8.26 aAB1	15.43 ± 1.45 aAB1	16.17 ± 1.33 aA1	18.85 ± 4.67 aB1	6.58 ± 3.71 aA1	5.47 ± 4.46 aA1	4.67 ± 3.93 aA1
	10	10.42 ± 10.34 aA1	9.77 ± 7.06 aAB1	14.26 ± 4.18 aA1	60.67 ± 6.69 bC1	52.11 ± 15.09 abC1	46.32 ± 3.87 aC1	34.39 ± 0.16 abB1	31.63 ± 15.85 abBC1	29.34 ± 10.11 abB1	4.09 ± 1.70 aA1	4.81 ± 2.94 aA1	4.77 ± 5.39 aA1
	14	24.40 ± 8.53 aAB1	40.21 ± 12.25 bAB1	34.89 ± 10.82 bAB1	82.04 ± 5.72 bC1	100.93 ± 42.54 bC1	83.63 ± 49.51 aB1	42.87 ± 15.84 bB1	66.6 ± 18.43 bBC1	79. 65 ± 39.50 bB1	7.52 ± 5.17 aA1	8.88 ± 6.75 aA1	10.08 ± 4.54 aA1
pH	0	5.64 ± 0.03 aA1	5.64 ± 0.03 aA1	5.64 ± 0.03 cA1	5.64 ± 0.03 aA1	5.64 ± 0.03 aA1	5.64 ± 0.03 aA1	5.64 ± 0.03 aA1	5.64 ± 0.03 aA1	5.64 ± 0.03 aA1	5.64 ± 0.03 bA1	5.64 ± 0.03 bA1	5.64 ± 0.03 bA1
	3	5.51 ± 0.03 aA1	5.51 ± 0.04 aA1	5.55 ± 0.07 bcAB1	5.73 ± 0.06 abB1	5.67 ± 0.08 aA1	5.74 ± 0.11 aB1	5.46 ± 0.04 aA1	5.49 ± 0.10 aA1	5.50 ± 0.06 aA1	5.53 ± 0.06 abA1	5.56 ± 0.17 bA1	5.49 ± 0.07 bA1
	7	5.22 ± 0.62 aA1	5.18 ± 0.54 aA1	5.05 ± 0.42 abcAB1	5.68 ± 0.04 abA1	5.25 ± 0.48 aA1	5.50 ± 0.17 aB1	5.53 ± 0.05 aA2	5.37 ± 0.22 aA12	5.01 ± 0.20 aAB1	4.85 ± 0.24 aA1	4.47 ± 0.38 aA1	4.37 ± 0.24 aA1
	10	4.77 ± 0.74 aA1	5.19 ± 0.11 aB1	4.92 ± 0.02 abAB1	5.76 ± 0.05 bA1	5.18 ± 0.59 aAB1	5.55 ± 0.17 aB1	5.46 ± 0.25 aA1	5.45 ± 0.10 aB1	5.23 ± 0.33 aB1	5.35 ± 0.20 abA2	4.38 ± 0.11 aA1	4.53 ± 0.35 aA1
	14	4.76 ± 0.63 aA1	5.15 ± 0.49 aA1	4.81 ± 0.41 aA1	5.91 ± 0.02 cB2	5.51 ± 0.06 aA1	5.43 ± 0.14 aA1	5.63 ± 0.18 aAB1	5.10 ± 0.99 aA1	5.44 ± 0.37 aA1	5.37 ± 0.48 abAB1	4.65 ± 0.09 aA1	4.73 ± 0.29 aA1

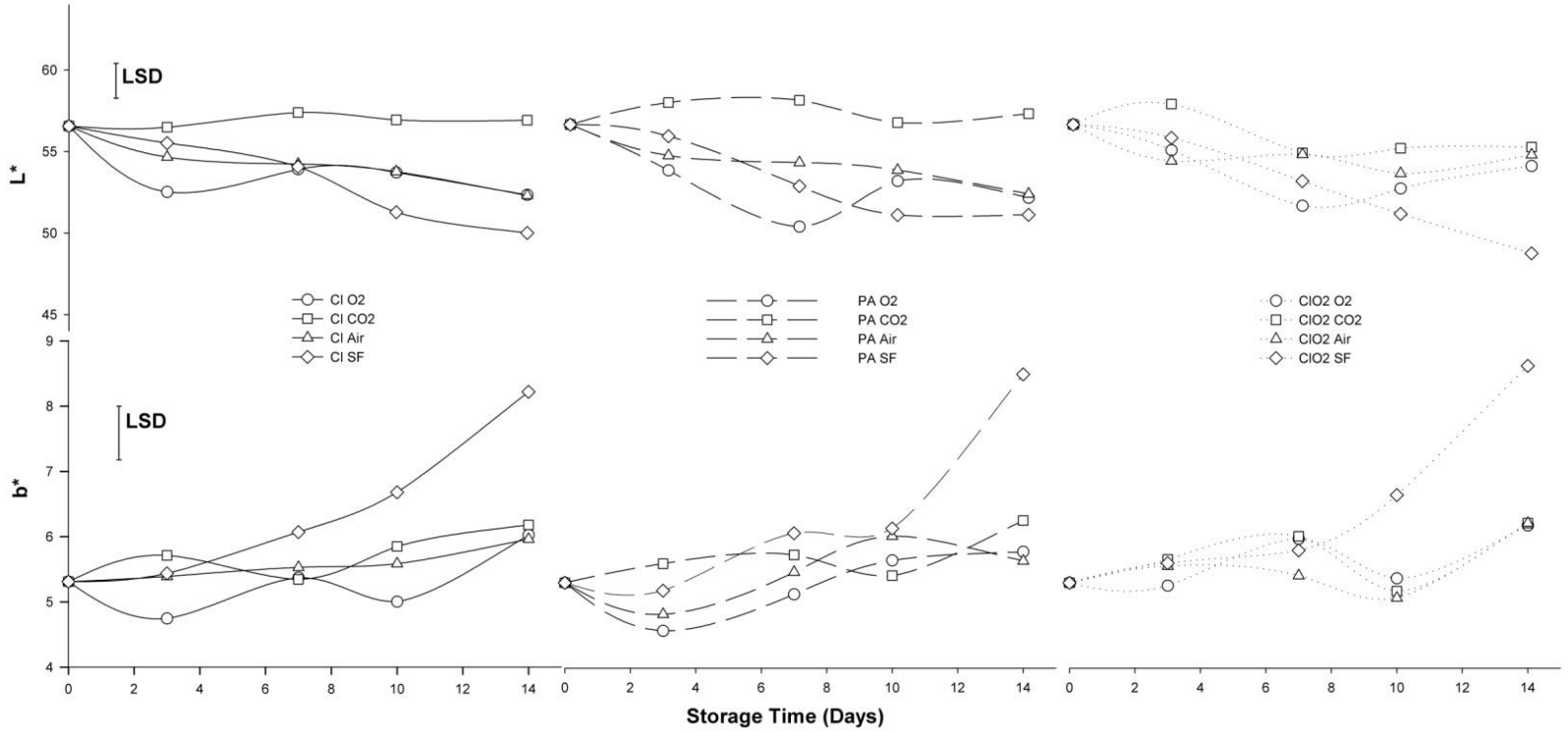
Table 9 (cont'd)
* Means sharing the same lowercase letter in the same column show no significant ($P < 0.05$) difference caused by time; means sharing the same uppercase letter in the same row for each sanitizer show no significant ($P < 0.05$) difference caused by gas composition; means sharing the same number in the same row for each gas composition show no significant ($P < 0.05$) difference caused by sanitizer.

5.2.3.2. Color

As shown in Figure 11, regardless of sanitizer, the in-package atmosphere CO2 best maintained the diced onions' L* value throughout storage while the snap-fit containers promoted the most ($P < 0.05$) darkening (interaction atmosphere and time; $P = 0.000$; Table 8). There was no difference between the effect of in-package atmospheres Air and O2 on the luminosity of the diced onions, which can be attributed to the same CO_2 contents reached in these packages throughout storage (Figure 10). Blanchard et al. (1996) reported that the higher the CO_2 content surrounding the diced onions, the lower the darkening since they observed that diced onions kept in a controlled atmosphere of 2 KPa O_2 + 15 KPa CO_2 had the least browning after 14 days compared to air and 2 KPa oxygen with 0, 5, and 10 kPa of CO_2. The b* value for diced onions (Figure 11) was also only significantly affected by in-package atmosphere throughout storage (interaction atmosphere and time; $P = 0.000$; Table 8). Although a change in color was observed in the diced onions of all packages, the diced onions in snap-fit containers were significantly ($P < 0.05$) more yellow over time. There was no difference in the yellowness of the diced onions between in-package atmospheres CO2, Air, and O2. Blanchard et al. (1996) also observed less yellowing in diced onions exposed to high CO_2 contents. Therefore, it seems that an in-package atmosphere rich in CO_2 can minimize both enzymatic browning and tissue yellowing in diced onions for all sanitizers studied. The sanitizers studied did not have an effect on either browning or tissue yellowing in diced onions ($P = 0.804$). The color of the diced onions was the same

throughout storage for all the sanitizers investigated although the capability of ClO2 to promote browning in some RTU products has been reported (Gómez-López et al., 2008).

Figure 11. Effect of atmosphere/sanitizer combinations on the L* and b* values of diced onions during 14 days of storage at 7 ^{o}C.



5.2.3.3. Weight loss

The weight loss of the diced onions increased as a function of time (P = 0.000; Table 8). At the end of storage, diced onions in the bags had a weight loss of ~3 % while those in the snap-fit containers had less than 1% weight loss, regardless of sanitizer (Table 9). The reason for this difference was the different water vapor permeability of the two packaging designs. The PLA bags had a water vapor transmission rate approximately 4 times greater than that of the PET snap-fit containers as shown in Table 7 and discussed in section 3.1.

The sanitizer used also affected the weight loss of the onions (P = 0.000; Table 8), with diced onions sanitized with PA having significantly lower (P < 0.05) weight loss than those sanitized with Cl or ClO2. The potential of ClO2 to damage tissue of RTU products has previously been reported in the literature (Gómez-López et al., 2008). An interaction between sanitizer and time (P = 0.005) was observed for diced onion weight loss, with the diced onions in CO2 or O2 sanitized with PA having the same weight loss at day 10. Regardless of packaging system and sanitizer, the change in weight of the diced onions throughout storage was not big enough to affect their marketability as shown in section 3.7. Moreover, Ohta et al. (2002) reported that weight losses which exceed 5% of the initial weight led to limited marketability and reduced retail value of fresh produce. Based on this criterion, the diced onions of all treatments would be marketable throughout the 14 days of storage.

5.2.3.4. pH

Acidification of all the packaged diced onions occurred throughout storage (P = 0.000), although differently depending on the in-package atmosphere and sanitizer (P = 0.000 and P = 0.001, respectively) (Table 8). Onions packaged in in-package atmospheres Air or CO2 had a significantly higher (P < 0.05) pH than those packaged in in-package atmospheres O2 or snap-fit

containers (Table 9). Therefore, it seems that a combination high CO_2 and low O_2 is necessary to reduce acidification in diced onions since the in-package atmospheres Air and O2 both had the same high CO_2 content throughout storage, but they differed in O_2 content during most of the storage period (Figure 10). Forney et al. (2012) also observed the least amount of acidification for diced onions in packages with the highest/lowest amounts of CO_2/O_2.

Onions sanitized with Cl had a significantly higher ($P < 0.05$) pH than those sanitized with PA or ClO2. The higher acidification of the diced onions sanitized with PA or ClO2 can be attributed to a greater amount of bacterial growth since diced onions sanitized with Cl had significantly lower ($P < 0.05$) mesophilic bacteria populations than those sanitized with PA or ClO2, regardless of the in-package atmosphere (Figure 9). In agreement, Forney et al. (2012) reported that increased microbial growth in diced onions caused a drop in their pH during storage. In contrast, Beerli, Vilas Boas and Piccoli (2004) observed that hydrogen peroxide decreased both counts of aerobic mesophiles and pH in packaged sliced onions compared to sodium dichloroisocianurate. Interactions between atmosphere and sanitizer were also observed with Cl SF and PA O2 resulting in less acidification of packaged diced onions (atmosphere and sanitizer $P = 0.011$; Table 8).

5.2.4. Effect of atmosphere/sanitizer combinations on sensory attributes of diced onions

Cl CO2 was the best in-package gas and sanitizer combination for enhancing the safety and quality of packaged diced onions as determined by the above microbiological and physico-chemical results. In order to determine the consumer acceptability of diced onions packaged in this gas composition/sanitizer combination, they were compared with freshly packaged diced onions for two weeks using descriptive sensory analysis and after 14 days using consumer acceptance sensory analysis. The results are presented below.

5.2.4.1. Descriptive sensory analysis

Trained panelists rated the off-odor, color, and overall appearance of diced onions packaged in Cl CO2 with significantly ($P < 0.05$) higher scores (poorer quality) than freshly packaged diced onions starting on day 7 of storage (Table 10). However, at day 14, panelists scored the color and overall appearance of the two different packaged diced onions as not being statistically different. The lack of differences detected at day 14 most likely was due to the use of a different batch of onions to obtain the freshly packaged diced onions used on each analysis day. However, a higher off-odor score was still obtained for the diced onions packaged in Cl CO2 compared to the freshly packaged diced onions. Because the majority of panelists (6/8) deemed diced onions packaged in Cl CO2 as acceptable for purchase after 14 days storage, this storage period was determined to be the acceptable shelf life of the diced onions packaged in Cl CO2 and chosen for the consumer panel to be performed in order to evaluate the consumer acceptance of onions packaged in Cl CO2. Other authors have also reported that the combination of chlorine sanitizer and elevated CO_2/ reduced O_2 atmosphere can lengthen the consumer acceptability for diced onions. Blanchard et al. (1996) reported that a trained panel scored higher (higher quality) chlorine-sanitized diced onions stored in 2 kPa O_2 + 10 kPa CO_2 and 2 kPa O_2 + 15 kPa CO_2 than those stored in air for both appearance and aroma after 14 days.

Table 10. Mean sensory scores for freshly packaged diced onions (I) and diced onions packaged in Cl CO2 (II) for 14 days at 7 °C.

	Off-odor		Color		Appearance		Acceptable for Purchase	
Day	I	II	I	II	I	II	I	II
0	1 aA*	1.13 aA	1.06 aA	1 aA	1.13 aA	1.06 aA	100%	100%
3	1.38 aA	1.25 aA	1.56 aA	1.13 aA	1.56 aA	1.13 abA	100%	100%
7	1.25 aA	2.5 bB	1.31 aA	2.06 bB	1.38 aA	2.13 cB	100%	87.5%
10	1.31 aA	2.69 bB	1.25 aA	2.25 bB	1.25 aA	2.19 cB	100%	87.5%
14	1.44 aA	3.19 bB	1.38 aA	2.06 bA	1.44 aA	2 bcA	100%	75%

*Means sharing the same lowercase letter in the same column are not significantly ($P < 0.05$) different. Means sharing the same uppercase letter in the same row are not significantly ($P < 0.05$) different. Number of trained panelists = 8. Three different 5-point scales were used (1 = no off odor, 5 = extreme off odor, rancid; 1 = bright white, 5 = darkened/discolored; 1 = fresh looking, typical onion appearance, 5 = mushy, dried out, wet on surface, spoiled).

5.2.4.2. Consumer acceptance sensory analysis

The results from the consumer panel (Table 11) show that panelists were not able to differ ($P < 0.05$) between the aroma, color, and overall appearance of diced onions packaged in Cl CO2 for 14 days and freshly packaged diced onions. In addition, the difference of purchase acceptability between the diced onions in the two packages was minimal. Over 97% of panelists said that the freshly packaged onions were acceptable for purchase versus 94% for diced onions packaged in Cl CO2, indicating that the evaluated gas composition/sanitizer combination can successfully maintain the accepted consumer quality of diced onions for 14 days. While the diced onions were deemed acceptable for purchase after 14 days of storage, the test was not carried out further because of the microbiological growth at that point in the experiment. Aerobic bacterial populations reached approximately 6 log CFU/g after 14 days in Cl CO2 (Figure 9), at which point the study was terminated.

Table 11. Mean sensory scores for freshly packaged diced onions (I) and diced onions packaged in Cl CO2 (II) for 14 days at 7 °C.

Aroma		**Color**		**Overall Appearance**		**Acceptable for Purchase?**	
I	II	I	II	I	II	I	II
6.04 A*	5.94 A	7.57 A	7.27 A	7.39 A	7.09 A	97.6% A	94.1% A

*Means sharing the same letter in the same row are not significantly ($P < 0.05$) different. Number of consumer panel = 85. Two 9-point hedonic score were used (1 = dislike extremely, 9 = like extremely).

CONCLUSIONS

The initial headspace gas composition and packaging material can significantly affect the quality and safety of celery sticks during the marketable period. Active MAP (CO_2-PLA) out-performed passive MAP (A-PLA) in maintaining celery stick quality but not safety. Conventional active MAP (CO_2-PLA) out-performed non-conventional active MAP (O_2-PLA and N_2-PLA) in maintaining the quality of the celery sticks. Non-conventional active MAP with 95 kPa O_2 suppressed the growth of *L. monocytogenes*, while a high concentration of CO_2 promoted growth during the first 10 d of storage. When comparing PLA and PP/PE as a packaging material, both impacted the quality of celery to varying degrees, but not *Listeria* growth. PLA allowed quicker development of gas compositions that better maintained the green color of the celery surface and cut ends, but increased cut end dehydration and weight loss. However, this weight loss was well within tolerable limits with celery turgor also unaffected during two weeks of storage. PLA retained ethanol that was released into the package headspace while PP/PE favored its escape from the package. The combination PLA and an initial gas composition of 95 kPa O_2 would be a viable packaging option when storing celery for up to one week due to the few quality changes and maintained *Listeria* populations. However, during longer storage, celery exposed to this initial gas composition showed more visual and textural defects along with high levels of ethanol.

Through descriptive sensory testing, the bio-based material was found to develop an adequate atmosphere to preserve color, texture and sensory quality in fresh-cut celery. In addition, the newly developed flexibility and color scales proved to be effective assessors for descriptive sensory analysis of fresh-cut celery, and could be applied to other fresh produce.

Both in-package atmosphere and sanitizer had a significant ($P < 0.05$) effect on the microbiological safety (*S.* Typhimurium) and quality (mesophilic bacteria, yeasts and molds) of packaged diced onions. Snap-fit containers, regardless of sanitizer, had diced onions with the most ($P < 0.05$) *S.* Typhimurium, mesophilic bacteria, yeasts and molds growth throughout storage, showing that an in-package atmosphere other than air can improve the microbiological safety and quality of diced onions. Regardless of in-package atmosphere, diced onions sanitized with ClO2 contained the most ($P < 0.05$) *S.* Typhimurium, and those sanitized with Cl had the least ($P < 0.05$) mesophilic bacteria, yeasts and molds, showing the different effectiveness of sanitizers on *S.* Typhimurium, mesophilic bacteria, and yeast and mold growth. The Cl and CO2 combination had diced onions with the lowest ($P < 0.05$) *S.* Typhimurium populations throughout storage, proving that in-package atmosphere and sanitizer interactions exist and can reduce *S.* Typhimurium growth in diced onions.

In-package atmosphere had a greater effect on the physico-chemical quality of packaged diced onions than sanitizer. While in-package atmosphere had a significant ($P < 0.05$) effect on all parameters evaluated (weight loss, headspace ethanol, pH, L*, b*, headspace O_2, and headspace CO_2), sanitizer, however, only had a significant effect on weight loss, pH, and headspace CO_2. Snap-fit containers, regardless of sanitizer, promoted more darkening, yellowing, and acidification in diced onions than other in-package atmospheres, while CO2 best maintained the L* value of diced onions and reduced yellowing and acidification. Snap-fit containers had lower headspace ethanol and less weight loss compared to CO2, both of which resulted from the different permeabilities of the packaging systems instead of their different atmospheres. Regardless of the in-package atmosphere, diced onions sanitized with Cl had significantly less ($P < 0.05$) CO_2 content and acidification than those sanitized with PA or ClO2,

and diced onions sanitized with PA had significantly lower ($P < 0.05$) weight loss than those sanitized with Cl or ClO2, indicating the greater damage to the onion tissue when ClO2 is used. In-package atmosphere and sanitizer interactions that enhance the physico-chemical quality of packaged onions were also identified, with the interactions Cl SF and PA O2 resulting in less acidification of packaged diced onions.

Considering all of the parameters researched, the current commercially used in-package atmosphere/sanitizer combination (ClO2 SF) was shown to not be the best choice for maintaining the safety and quality of packaged diced onions during a 2 week storage at 7 °C. Conversely, Cl CO2 was the best in-package atmosphere and sanitizer combination for enhancing the safety and quality of packaged diced onions. Diced onions packaged in this in-package atmosphere and sanitizer combination would be acceptable for purchase after 14 days as verified by both a trained panel and a consumer panel. This period would also be acceptable in terms of microbiological counts for mesophilic bacteria and yeasts and molds for diced onions.

FUTURE WORK

These studies show the importance of researching the impact of packaging material, in-package atmosphere, sanitizer, and their combination on the quality and safety of fresh-cut produce. More research on the topic would help aid in the understanding of how packaging material, in-package atmosphere, sanitizer, and their combination affect the quality and safety of fresh-cut produce, and could include the following:

- A sensory evaluation would be needed to determine the impact of in-package ethanol concentration on the consumer acceptance of packaged celery sticks.
- Researching the effects of in-package atmosphere and sanitizer combinations on the safety and quality of celery sticks at temperatures other than 7 °C.
- Examination of the effects of alternative in-package atmospheres and sanitizers on the safety and quality of fresh-cut celery.
- Investigation of the effects of real life temperature storage (fluctuating temperatures) and in-package atmospheres and sanitizers on the safety and quality of celery sticks.
- Studying the impact of in-package atmosphere and sanitizer combinations on the growth of other common pathogenic microorganisms on fresh-cut celery to determine the effectiveness of those combinations at helping to mitigate the damage caused by contamination.
- Applying the newly developed celery sensory scales to other fresh produce items.
- Studying the effects of other in-package atmospheres and sanitizers on the flexibility and color change of fresh-cut celery
- Qualify the newly developed flexibility and color scales with fresh-cut celery stored at other or fluctuating temperatures

- Develop more standardized color scales specific to fresh produce degradation
- Researching the effects of in-package atmosphere and sanitizer combinations on the safety and quality of diced onions at temperatures other than 7 °C.
- Examination of the effects of alternative in-package atmospheres and sanitizers on the safety and quality of diced onions.
- Investigation of the effects of real life temperature storage (fluctuating temperatures) and in-package atmospheres and sanitizers on the safety and quality of diced onions.
- Studying the impact of in-package atmosphere and sanitizer combinations on the growth of other common pathogenic microorganisms on diced onions to determine the effectiveness of those combinations at helping to mitigate the damage caused by contamination.
- Determining the effect of in-package atmosphere and sanitizer combinations on the safety and quality of other fresh-cut produce.

BIBLIOGRAPHY

BIBLIOGRAPHY

Aguado, V., Vitas, A.I., García-Jalón, I., 2004. Characterization of *Listeria monocytogenes* and *Listeria innocua* from a vegetable processing plant by RAPD and REA. International Journal of Food Microbiology. 90, 341-347.

All About Onions, 2011. The National Onion Association. http://www.onions-usa.org/all-about-onions/consumption.

Almenar, E., Samsudin, H., Auras, R., Harte, B., Rubino, M., 2008. Postharvest shelf life extension of blueberries using a biodegradable package. Food Chemistry. 100(1), 120-127.

Almenar, E., Auras, R., 2010. Permeation, sorption, and diffusion in poly(lactic acid), in Auras, R., Lim, L-T, Selke, S.E.M., Tsuji, H. (Eds.), Poly(lactic acid): Synthesis, Structure, Properties and Applications. Wiley and Sons, New York. 155-179.

Almenar, E., Samsudin, H., Auras, R., Harte, J., 2010. Consumer acceptance of fresh blueberries in bio-based packages. Journal of Science Food Agriculture. 90(7), 1121-1128.

Almenar, E., Gartner, H., 2015. Active Packaging – An Introduction. In: Wani, A.A., Singh, P., Laub, S.S., Langowski, H-C (Eds.), Modified Atmosphere, Intelligent and Active Packaging: Science and Technology. Wiley-Blackwell Publishing Limited, Oxford (In press).

Allende, A., Jacxsens, L., Devlieghere, F., Debevere, J., Artés, F., 2002. Effect of superatmospheric oxygen packaging on sensorial quality, spoilage, and *Listeria monocytogenes* and *Aeromonas caviae* growth in fresh processed mixed salads. Journal of Food Protection. 65, 1565-1573.

Allende, A., Luo, Y., McEvoy, J.L., Artés, F., Wang, C.Y., 2004. Microbial and quality changes in minimally processed baby spinach leaves stored under super atmospheric oxygen and modified atmosphere conditions. Postharvest Biology and Technology. 33, 51-59.

Alvarez, M.D.; Saunders, D.E.J.; Vincent, J.F.V.; Jeronimidis, G., 2001. An Engineering Method to Evaluate the Crisp Texture of Fruit and Vegetables. Journal of Texture Studies. 31, 457–473.

Amanatidou, A., Smid, E.J., Gorris, L.G.M., 1999. Effect of elevated oxygen and carbon dioxide on the surface growth of vegetable-associated micro-organisms. Journal of Applied Microbiology. 86, 429-438.

Artés, F., Gómez, P., Aguayo, E., Escalona, V., Artés-Hernández, F., 2009. Sustainable sanitation techniques for keeping quality and safety of fresh-cut plant commodities. Postharvest Biology and Technology. 51, 287-296.

Artés-Hernandez, F., Rivera-Cabrera, F., Kader, A.A., 2007. Quality retention and potential shelf-life of fresh-cut lemons as affected by cut type and temperature. Postharvest Biology and Technology. 43, 245-254.

Beerli, K.M.C., Boas, V., de Barros, E.V., Piccoli, R.H., 2004. Effect of sanitizers on the microbial, physical and physical-chemical characteristics of fresh-cut onions (*Allium cepa* L.). Ciência e Agrotecnologia. 28, 107-112.

Bennik, M.H.J., Peppelenbos, H.W., Nguyen-the, C., Carlin, F., Smid, E.J., Gorris, L.G.M., 1996. Microbiology of minimally processed, modified-atmosphere packaged chicory endive. Postharvest Biology and Technology. 9, 209-221.

Bett, K.L., 2002. Evaluating Sensory Quality of Fresh-cut Fruits and Vegetables. CRC Press. Boca Raton, FL.

Blanchard, M., Castaigne, F., Willemot, C., Makhlouf, J., 1996. Modified atmosphere preservation of freshly prepared diced yellow onion. Postharvest Biology and Technology. 9, 173-185.

Block, E., Calvey, E.M., Gillies, C.W., Gillies, J.Z., Uden, P., 1997. Peeling the onion. Organosulfur and selenium phytochemicals in genus Allium plants. In: Johns, T., Romeo, J.T. (Eds.), Functionality of Food Phytochemicals. Plenum Press, New York. 1-30.

Buta, J.G., Moline, H.E., 1998. Methyl Jasmonate Extends Shelf-life and reduces microbial contamination of fresh cut celery and peppers. Journal of Agriculture Food Chemistry. 46, 1253-1256.

Caleb, O.J, Mahajan, P.V., Al-Said, F.A., Opara, U.L., 2013. Modified Atmosphere Packaging Technology of Fresh and Fresh-cut Produce and the Microbial Consequences—A Review. Food Bioprocess Technology. 6, 303-329.

Cantwell, M.I., Suslow, T.V., 2002. Postharvest handling systems: fresh-cut fruits and vegetables, in Kader, A.A. (Ed.), Postharvest Technology of Horticultural Crops. University of California, Berkeley, California, Publications. 3311, 445–463.

Carrasco, E., Pérez-Rodríguez, F., Valero, A., García-Gimeno, R.M., Zurera, G., 2008. Growth of *Listeria monocytogenes* on shredded, ready-to-eat iceberg lettuce. Food Control. 19, 487-494.

Charalambides, M.N., Williams, J.G., Chakrabarti, S., 1995. A study of the influence of ageing on the mechanical properties of Cheddar cheese. Journal of Materials Science. 30(16), 3959-3967.

Chen, L. & Opara, U.L., 2013. Texture measurement approaches in fresh and processed foods - A review, Food Research International. 51(2), 823-835.

Cisneros-Zevallos, L., Saltveit, M.E., Krochta, J.M., 1995. Mechanism of surface white discoloration of peeled (minimally processed) carrots during storage. Journal of Food Science. 60, 320-323.

Cordano, A.M., Jacquet, C., 2009. *Listeria monocytogenes* isolated from vegetable salads sold at supermarkets in Santiago Chile: Prevalence and strain characterization. International Journal of Food Microbiology. 132, 176-179.

Day, B.P.F., 1989. Modified atmosphere packaging of selected prepared fruits and vegetables. Technical memorandum, Campden Food and Drink Research Association. 524, 65.

De Roeck, A., Mols, J., Duvetter, T., Van Loey, A., & Hendrickx, M., 2010. Carrot texture degradation kinetics and pectin changes during thermal versus high-pressure/high-temperature processing: A comparative study. Food Chemistry. 120, 1104–1112.

Escalona, V.H., Verlinden, B.E., Geysen, S., Nicolaï, B.M., 2006. Changes in respiration of fresh-cut butterhead lettuce under controlled atmospheres using low and superatmospheric oxygen conditions with different carbon dioxide levels. Postharvest Biology and Technology. 39, 48-55.

Evans, K., Brutcher, L., Konishi, B., Barritt, B., 2010. Correlation of sensory analysis with physical textural data from a computerized penetrometer in the Washington State University Apple breeding program. HortTechnology. 20, 1026-1029.

Everard, C.D., O'Callaghan, D.J., O'Kennedy, B.Y., O'Donell, C.P., Sheehan, E.M., Delahunty, C.M., 2007. A three-point bending test for prediction of sensory texture in processed cheese. Journal of Texture Studies. 38, 438-456.

Everson, H.P., Waldron, K.W., Geeson, J.D., Browne, K.M., 1992. Effects of modified atmospheres on textural and cell-wall changes of asparagus during shelf-life. International Journal of Food Science and Technology. 27, 187-199.

Farber, J.M., Wang, S.L., Cai, Y., Zhang, S., 1998. Changes in populations of *Listeria monocytogenes* inoculated on packaged fresh-cut vegetables. Journal of Food Protection. 61, 192-195.

Farris, S., Gobbi, S., Torreggiani, D., & Piergiovanni, L., 2008. Assessment of two different rapid compression tests for the evaluation of texture differences in osmoair-dried apple rings. Journal of Food Engineering. 88, 484–491.

Fishman, S., Rodov, V., Ben-Yehoshua, S., 1996. Mathematical model for perforation effect on oxygen and water vapor dynamics in modified atmosphere packages. Journal of Food Science. 61, 956-961.

Forney, C.F., Jordan, M.A., Doucette, C., Grant, R., 2012. Influence of packaging on loss of aroma and quality of diced red onions. Acta Horticulturae. 934, 523-530.

Francis, G.A., O'Beirne, D., 2001. Effects of vegetable type, package atmosphere and storage temperature on growth and survival of *Escherichia coli* O157:H7 and *Listeria monocytogenes*. Journal of International Microbiology and Biotechnology. 27, 111-116.

Fresh Cut Magazine, 2008. Some segments see triple-digit increases. http://freshcut.com/index.php/magazine/article/Some-segments-see-triple-digit-increases.

Gaul, L.K., Farag, N.H., Shim, T., Kingsley, M.A., Silk, B.J., Hyytia-Trees, E., 2013. Hospital-acquired listeriosis outbreak caused by contaminated diced celery - Texas 2010. Clinical Infectious Diseases. 56, 20-26.

Gómez, P. A., & Artés, F., 2004. Controlled atmospheres enhance postharvest green celery quality. Postharvest Biology and Technology. 34, 203-209.
Gómez, P. A., & Artés, F., 2005. Improved keeping quality of minimally fresh processed celery sticks by modified atmosphere packaging. LWT-Food Science and Technology. 38, 323-329.

Gómez-López, V.M., Ragaert, P., Jeyachchandran, V., Debevere, J., Devlieghere, F., 2008. Shelf-life of minimally processed lettuce and cabbage treated with gaseous chlorine dioxide and cysteine. International Journal of Food Microbiology. 121, 74-83.

González, J., Ferrer, A., Oria, R., Salvador, M.L., 2008. Determination of O_2 and CO_2 transmission rates through microperforated films for modified atmosphere packaging of fresh fruits and vegetables. Journal of Food Engineering. 86, 194-201.

González-Buesa, J., Page, N., Kaminski, C., Ryser, E., Beaudry, R., Almenar, E., 2014. Effect of non-conventional atmospheres and bio-based packaging on the safety and quality of fresh-cut celery (*Apium graveolens* L.). Postharvest Biology and Technology. 93, 29-37.

González-Buesa, J., Page, N., Kaminski, C., Ryser, E.T., Beaudry, E.T. and Almenar, E., 2013 Influence of Initial Gas Composition and Packaging Material on the Safety and Quality of Fresh-cut Celery (Apium graveolens L.). Postharvest Biology and Technology.

González-Buesa, J., Arias, E., Salvador, M.L., Oria, R., Ferrer-Mairal, A., 2011. Suitability for minimal processing of non-melting clingstone peaches. International Journal of Food Science and Technology. 46, 819-826.

Gorny, J.R., Hess-Pierce, B., Kader, A.A., 1998. Effects of fruit ripeness and storage temperature on the deterioration rate of fresh-cut peach and nectarine slices. HortScience. 33, 110-113.

Hadjok, C., Mittal, G.S., Warriner, K., 2008. Inactivation of human pathogens and spoilage bacteria on the surface and internalized within fresh produce by using a combination of ultraviolet light and hydrogen peroxide. Journal of Applied Microbiology. 104, 1014-1024.

Howard, L.R., Yoo, K.S., Pike, L. M., Miller, J.G., 1994. Quality changes in diced onions stored in film packages. Journal of Food Science. 59, 110-112.

Imbeni, V, Atkins, A.G., Heronimidis, J. and Yeo, J., 2001. Rate and temperature dependence of the mechanical properties of cheddar cheese. In: Proceedings of 10th International Conference on Fracture.

Iqbal, T., Rodrigues, F.A., Mahajan, P.V., Kerry, J.P., Gil, L., Manso, M.C., Cunha, L.M., 2008. Effect of minimal processing conditions on respiration rate of carrots. Journal of Food Science. 73, E396-402.

Jacxsens, L., Devlieghere, F., Van der Steen, C., Debevere, J., 2001. Effect of high oxygen modified atmosphere packaging on microbial growth and sensorial qualities of fresh-cut produce. International Journal of Food Microbiology. 71, 197-210.

Jacxsens, L., Devlieghere, F., Ragaert, P, Vanneste, E., Debevere, J., 2003. Relation between microbiological quality, metabolite production and sensory quality of equilibrium modified atmosphere packaged fresh-cut produce. International Journal of Food Microbiology. 83, 263-280.

James, B., Young, A., Smith, B., Kim, E., Wilson, A., & Morgenstern, M. P., 2011. Texture changes in bolus to the "point of swallow" - Fracture toughness and back extrusion to test start and end points. Procedia Food Science. 1, 632–639.

Jol, S. Kassianenko, A., Wszol, K., Oggel, J., 2006. Issues in time and temperature abuse of refrigerated food. Food Safety. 11, 32-35, 78.

Joo, M., Lewandowski, N., Auras, R., Harte, J., Almenar, E., 2011. Comparative shelf life study of blackberry fruit in bio-based and petroleum-based containers under retail storage conditions. Food Chemistry. 126, 1734-1740.

Joo, M., Lewandowski, N., Auras, R., Harte, J., Almenar, E., 2012. Corrigendum to "Comparative shelf life study of blackberry fruit in bio-based and petroleum-based containers under retail storage conditions" Food Chemistry. 126, 1734–1740.

Kader, A.A., Lipton, W.J., Morris, L.L. 1973. Systems of scoring quality of harvested lettuce. Horticulture Science. 8, 408-409.

Kallander, K.D., Hitchins, A.D., Lancette, G.A., Schmieg, J.A., Garcia, G.R., Solomon, H.M., Sofos, J.N., 1991. Fate of *Listeria monocytogenes* in shredded cabbage stored at 5 and 25 ºC under a modified atmosphere. Journal of Food Protection. 54, 302-304.

Kamyab, I. Chakrabarti, S. and Williams, J.G., 1998. Cutting cheese with wire. Journal of Materials Science. 33(11), 2763-2770.

Koutsimanis, G., Getter, K., Behe, B., Harte, J., Almenar, E., 2012. Influences of packaging attributes on consumer purchase decisions for fresh produce. Appetite. 59, 270-280.

Kwak, T.Y., Kim, N.H., Rhee, M.S., 2011. Response surface methodology-based optimization of decontamination conditions for *Escherichia coli* O157:H7 and *Salmonella* Typhimurium on fresh-cut celery using thermoultrasound and calcium propionate. International Journal of Food Microbiology. 150, 128-135.

Lim, L.T., Auras, R., Rubino, M., 2008. Processing technologies for poly(lactic acid). Progress in Polymer Science. 33, 820-852.

Liu, F., Li, Y., 2006. Storage characteristics and relationships between microbial growth parameters and shelf life of MAP sliced onions. Postharvest Biology and Technology. 40, 262-268.

Loazia-Velarde, J.G., Mangrich, M.E., Campos-Vargas, R., Saltveit, M.E., 2003. Heat Shock reduces browning of fresh-cut celery petioles. Postharvest Biology and Technology. 27, 305-311.

Lu, Z., Yu, Z., Gao, X., Lu, F., Zhang, L., 2005. Preservation effects of gamma irradiation on fresh-cut celery. Journal of Food Engineering. 67, 347-351.

Lundén, J., Vanhanen, V., Kotilainen, K., Hemminki, K., 2014. Retail food stores' internet-based own-check databank records and health officers' on-site inspection results for cleanliness and food holding temperatures reveal inconsistencies. Food Control. 35, 79-84.

Mannapperuma, J.D., Zagory, D., Singh, P., Kader, A.A., 1989. Design of polymeric packages for modified atmosphere storage of fresh produce. In: Fellman, J.K. (Ed.), Proceedings of the Fifth International Controlled Atmosphere Research Conference, vol. 2 – Other Commodities Storage Recommendations, 225-233.

Martínez, J.A., Chiesa, A., Tovar, F., Artés, F., 2005. Respiration rate and ethylene production of fresh cut lettuce as affected by cutting grade. Agric. Food Sci. 14, 354-361.

Martínez-Hernández, G.B., Navarro-Rico, J., Gómez, P.A., Otón, M., Artés, F., Artés-Hernández, F., 2015. Combined sustainable sanitizing treatments to reduce *Escherichia coli* and *Salmonella* Enteriditis growth on fresh-cut Kailan-hybrid broccoli. Food Control 47, 120-125.

Martínez-Sánchez, A., Allende, A., Bennett, R.N., Ferreres, F., Gil, M.I., 2006. Microbial, nutritional and sensory quality of rocket leaves as affected by different sanitizers. Postharvest Biology and Technology 42, 86-97.

Massman, W.J., 1998. A review of the molecular diffusivities of H_2O, CO_2, CH_4, CO, O_3, SO_2, NH_3, N_2O, NO, and NO_2 in air, O_2 and N_2 near STP. Atmospheric Environment 32, 1111-1127.

Meilgaard, M., Civille, G.V., Carr, B.T., 1999. Sensory Evaluation Techniques 3rd edition. CRC Press. Boca Raton, FL. 43-56, 195-208.

Meullenet, J., Lyon, B.G., Carpenter, J.A., Lyon, C.E., 1997. Relationship between sensory and instrumental texture profile attributes. Journal of Sensory Studies. 13, 77-93.

Ohta, H., Shiina T., Sasaki, K., 2002. Dictionary of freshness and shelf life of food. Tokyo: Science Forum Co. Ltd.

PANTONE, 2013. About PANTONE: Who we are. <http://www.pantone.com/pages/pantone.aspx?pg=19306>

Park, W.P., Lee, D.S., 1995. Effect of chlorine treatment on cut water cress and onion. Journal of Food Quality. 18, 415-424.

Pelag, M., 1980. Theoretical analysis of the relationship between mechanical hardness and its sensory assessment. Journal of Food Science 45, 1156-1160.

Piercey, M.J., Mazzanti, G., Budge, S.M., Delaquis, P.J., Paulson, A.T., Truelstrup Hansen, L., 2012. Antimicrobial activity of cyclodextrin entrapped allyl isothiocyanate in a model system and packaged fresh-cut onions. Food Microbiology. 30, 213-218.

Poste, L.M., Mackie, D.A., Butler, G. and Larmond, E., 1991. Laboratory Methods for Sensory Analysis of Food. Canada Communications Group-Publishing Centre, Ottawa, Canada, 61-68.

Prakash, A., Inthajak, P., Huibregtse, H., Caporaso, F., Foley, D.M., 2000. Effects of low-dose gamma irradiation and conventional treatments of shelf life and quality characteristics of diced celery. Journal of Food Science. 65(6),1070-1075.

Raffo, A., Sinesio, F., Moneta, E., Nardo, N., Peparaio, M., Paoletti, F., 2006. Internal quality of fresh and cold stored celery petioles described by sensory profile, chemical and instrumental measurements. European Food Research and Technology. 222, 590-599.

Rasal, R.M., Janorkar, A.V., Hirt, D.E., 2010. Poly(lactic acid) modifications. Progress in Polymer Science. 35, 338-356.

Rizzo, V., Muratore, G., Russo, M.A. & Belligno, A., 2011. Shelf Life Study of Fresh Celery (Apium graveolens L.) Grown under Different Nitrogen Fertilization Treatments. Journal of Food Science. 75(4), S225-S232.

Rizzo, V., & Muratore, G., 2009. Effects of packaging on shelf life of fresh celery. Journal of Food Engineering. 124-128.

Robertson, G.L., 2013. Food packaging: principles and practice. (3rd ed.). CRC Press, Boca Raton, FL, USA.

Rizzo, V., Muratore, G., 2009. Effects of packaging on shelf life of fresh celery. Journal of Food Engineering. 90, 124-128.

Robinson, J.E., Browne, K.M., Burton, W.G., 1975. Storage characteristics of some vegetables and soft fruits. Ann. Applied Biology. 81, 399-408.

Rocculi, P., Romani, S., Dalla Rosa, M., 2005. Effect of MAP with argon and nitrous oxide on quality maintenance of minimally processed kiwifruit. Postharvest Biology and Technology. 35, 319-328.

Rojo, F.J., & Vincent, J. F.V., 2009. Objective and subjective measurement of the crispness of crisps from four potato varieties. Engineering Failure Analysis. 16, 2698–2704.

Saltveit, M., Mangrich, M., 1996. Using density measurements to study the effect of excision, storage, abscisic acid and ethylene on pithiness of celery petioles. Journal of the American Society for Horticultural Science. 1, 137-141.

Saltveit, M.E., 1997. A summary of CA and MA requirements and recommendations for harvested vegetables, in: Proceedings of the 7th International Controlled. Atmosphere Conference. Davis, CA, 98-117.

Selman, J.D., 1993. Practical experiences of sensory investigations into the packaging of vegetables. Packaging Technology and Science. 6, 183-193.

Scully, A., 2009. Active Packaging, in Yam, K.L. The Wiley Encyclopedia of Packaging Technology. Wiley and Sons, New York, 2-9.

Sila, D.N., Smout, C., Vu, T.S., & Hendrickx, M.E., 2004. Effects of high-pressure pretreatment and calcium soaking on the texture degradation kinetics of carrots during thermal processing. Journal of Food Science. 69, E205–E211.

Silveira, A.C., Conesa, A., Aguayo, E., Artés, F., 2008. Alternative sanitizers to chlorine for use on fresh-cut 'Galia' (Cucumis melo var. catalupensis) melon. Journal of Food Science. 73, M405–M411.

Stone, H., Sidel, J., Oliver, S., Woolsey, A., Singleton, R.C., 1974. Sensory evaluation by quantitative descriptive analysis. Food Technology. 8, 24-32.

Suslow, T., Cantwell, M., 2000. Celery: Recommendations for Maintaining Postharvest Quality. Produce facts. Department of Vegetable Crops, California, USA.

Sy, K.V., Murray, M.B., Harrison, M.D., Beuchat, L.R., 2005. Evaluation of gaseous chlorine dioxide as a sanitizer for killing *Salmonella*, *Escherichia coli* O157: H7, *Listeria monocytogenes*, and yeasts and molds on fresh and fresh-cut produce. Journal of Food Protection. 68, 1176-1187.

Tatsumi, Y., Watada, A.E., Wergin, W.P., 1991. Scanning Electron-Microscopy of Carrot Stick Surface to Determine Cause of White Translucent Appearance. Journal of Food Science. 56, 1357-1359.

Tharanathan, R.N., 2003. Biodegradable films and composite coatings: past, present and future. Trends in Food Science and Technology. 14, 71-78.

Toivonen, P.M.A., Brummell, D.A. 2008. Biochemical Bases of Appearance and Texture Changes in fresh-cut fruit and vegetables. Postharvest Biology and Technology. 48, 1-14.

United Fresh Foundation, 2010. FreshFacts® on Retail. Whole and Fresh Cut Produce Trends: Q3 2010.

Vandamm, J.P., Li, D., Harris, L.J., Schaffner, D.W., Danyluk, M.D., 2013. Fate of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* on fresh-cut celery. Food Microbiology. 34, 151-157.

Viña, S.Z., Chaves, A.R., 2003. Texture changes in fresh cut celery during refrigerated storage. Journal of Science Food Agriculture. 83, 1308-1314.

Viña, S.Z., Chaves, A.R., 2006. Antioxidant responses in minimally processed celery during refrigerated storage. Food Chemistry. 94, 68-74.

Viña, S.Z., López Osornio, M.M., Chaves, A.R., 2007. Quality changes in fresh-cut celery as affected by heat treatment and storage. Journal of Science Food Agriculture 87, 1400-1407.

Voisey, P.W., 1971. Modernization of texture instrumentation. Journal of Texture Studies. 2:129-195.

Watada, A.E., Qi, L., 1998. Quality of Fresh-cut Produce. Postharvest Biology and Technology. 15: 201-205.

Yang, E., Fan, L., Jiang, Y., Doucette, C., Fillmore, S., 2011. Antimicrobial effects of a commercial fermentation product on fresh-cut onions. Journal of Food Protection 74, 470-474.

Yasuda, H., 1975. Units of gas permeability constants. Journal of Applied Polymer Science 19, 2529-2536.

Zaulia, O., Azhar, M.N., Razali, M., Nur Aida, M.P., Bizura Hasida, M.R., Habsah, M., Hairiyah, M., Rohaya, M.A., Zaipun , M.Z., Tham, S.L., 2013. Active modified atmosphere packaging using ZP Ageless to control cut surface browning of peeled onion. Acta Horticulturae 1012, 979-986.

Zhang, L., Lu, Z., Yu, Z., Gao, X., 2004. Preservation of fresh-cut celery by treatment of ozonated water. Food Control. 16: 279-283.